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## PROTOZOOLOGY.

### (a) AMOEBIASIS.

(171) DARLING (S. T.). **Entamebic Dysentery in the Dog.**—*Proc. Med. Assoc. Isthmian Canal Zone for the Half-Year, Apr. to Sept. 1913* [1915.] Vol. 6. Part 1. pp. 60–62.

This appears to be the first case recorded of naturally acquired entamebic dysentery in the dog.

The subject was a large deerhound which had been in the Panama Canal Zone for several months and had just been brought into the laboratory for observation in connection with hookworms. On admission it was suffering from blood-stained diarrhoea and great abdominal pain. Faeces on examination were found sometimes to contain trophozoites. The animal became very emaciated and died after 11 days.

Post-mortem examination showed that the mucous membrane of the colon was dotted all over with innumerable minute red punctate superficial erosions, but no large ulcers. Smears always contained entamoebae in which at times red blood cells were seen. The ileum contained hundreds of hook-worms, but smears of the mucous membrane contained no entamoebae although a few were seen in sections on the surface.

On histological examination the minute red ulcers on the mucous membrane of the colon were seen to be infiltrated with leucocytes and entamoebae. The superficial portion of the ulcers was denuded of epithelium, and the surrounding surface was covered with mucus which contained many trophozoites apparently vegetating in it. Clumps of entamoebae could be seen in places making their way into the intestinal crypts. The invasion was very superficial and the submucosa and muscularis were not involved. The lesions were thus not like those seen in the cat experimentally infected per rectum with the trophozoites of *E. tetragena* of man. This suggests that the lesions were caused by a different species of entamoeba.

*Description of the trophozoite.*—In the wet state it measured from 16 to 32 microns in diameter; the majority contained no red blood corpuscles but some contained up to 8 or 10. The ectosarc was clear and well differentiated from the endoplasm which was granular and

(C296) Wt. P11/85. 1,000. 9.16. B.&F.Ltd. Gp.11/5.

contained vacuoles. The nucleus and usually its karyosome were easily detected. When stained *intra vitam* with gentian violet the peripheral chromatin of the nucleus was displayed as many fine particles, and the centriole and karyosome were distinctly shown. With phosphotungstic acid haematoxylin, and iron haematoxylin, the trophozoites stained well and the nucleus was seen to be made up of rather fine chromatin granules. The centriole was not uncommonly eccentrically placed while the karyosome appeared as a finely granular ring.

Specimens dry fixed and stained with Giemsa and differentiated with ammoniated ethyl alcohol present a picture not unlike *E. tetragena*, except that the karyosome is quite different from that usually seen in the latter. It is smaller and more dense, its band is broader and it is never seen in the form of a reticulum, nor as granules scattered through the nucleus as in *E. tetragena*. Specimens in films wet fixed and stained with haematoxylin gave the following average diametrical measurements in microns. Trophozoite 21 to 28, nucleus 5·5, karyosome 2·4, centriole 0·7.

All the trophozoites were of the large vegetative type usually seen in active lesions. Their appearance was in general like that of *E. tetragena* except for important differences in structure of the nucleus. Cysts and other perpetuating forms were not discoverable. A puppy fed with material containing trophozoites six hours after the death of the host did not contract the disease. Rectal inoculations were not made.

From the characters enumerated above the author believes that the entamoeba found in the dog differs from *E. tetragena* and *E. coli* which cause lesions in the cat and man, and provisionally proposes the name *E. venaticum* for this new species.

(172) Ware (F.). **The Possibility of Amoebic Dysentery in the Dog, and its Treatment with Emetin.**—*Jl. Comp. Path. & Therap.* 1916. June. Vol. 29. Pt. 2. pp. 126–130.

Fresh faeces were examined microscopically from a rather acute case of dysentery in a bitch. This animal belonged to a pack of foxhounds kept at a hill station in India, in which dysentery was stated to have been a considerable source of trouble for some years past. In these smears of faeces one or more amoebae, which were pronounced to be extremely like *Entamoeba hystolytica*, the cause of amoebic dysentery in man, were present in at least every other field of the microscope. Six other animals were also clinically affected but not to so serious an extent as in the above case. No microscopic examination was made of their faeces.

All the above cases were treated with injections of emetin hydrochloride in daily doses of ½ or 1 grain until the symptoms abated. All quickly recovered except one case, where the animal had been ill for ten months. Post-mortem examination of the latter animal indicated that the large intestine was affected with numerous small ulcers, the stomach was considerably inflamed, and the liver enlarged. No search for amoebae seems to have been made.

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(173) Thomson (J. Gordon) & Thomson (D.). **Some Observations on the Effect of Emetine Administration on the Free Vegetative Forms and Cysts of *Entamoeba histolytica* and *Entamoeba coli*.**—*Jl. Roy. Army Med. Corps.* 1916. June. Vol. 26. No. 6. pp. 683–694. With 1 plate.

Summary of Conclusions.

"(1) If an amoebic case receive a continuous course of treatment with emetine of not less than a total of seven to ten grains of the drug administered in grain doses daily for a week, it is probable that he will never become a carrier of cysts. In order, however, to make certain of this, it is better to carry the treatment further. Such treatment also prevents relapses.

"(2) Cysts of *E. histolytica* can be cleared out of a patient after six grains of emetine, but, to make absolutely certain of this, it is better to exceed this dose. This treatment must be combined with saline purgatives, so as to get free daily action of the bowels.

"(3) A case of amoebic dysentery inefficiently treated with less than seven grains of emetine is likely to become highly dangerous as a carrier of cysts. One or two small doses may act beneficially and get rid of active symptoms, such as the passage of blood and mucus, but it may at the same time stimulate the formation of large numbers of cysts. The cessation of treatment on the disappearance of active symptoms is fraught with danger, both to the patient, and subsequently, to others.

"(4) All patients known to have blood and mucus in their stools and to have received less than an efficient total quantity of emetine ought to be examined for the presence of cysts in the faeces, as it is highly probable they have thereby become dangerous carriers.

"(5) The transmission of amoebic dysentery is undoubtedly due to the ingestion of cysts. This is brought about by the contamination of food, fingers, or flies. Cysts may also be carried along with sand particles by wind.

"(6) The powers of resistance of cysts outside the body are considerable, and this should be borne in mind by all responsible for sanitary prophylactic measures against dysentery."

(b) Anaplasmosis.

(174) Walker (J.). **Some Observations in connection with the Immunisation of Cattle against South African Redwater and Genuine Gallsickness (Anaplasmosis).**—*Union of South Africa. Dept. of Agric. 3rd and 4th Reports of the Director of Veterinary Research.* 1915. Nov. pp. 501–526. With 1 chart. Pretoria: Govt. Printing & Stationery Office.

The author begins this article by giving a complete review of the literature dealing with anaplasmosis.

(1) Observations in Connection with the Transmission of Redwater.

A series of 24 inoculations and immunity tests with redwater-gallsickness blood was performed on cattle, the results of which are summarised as follows:—

"1. The inoculation of susceptible cattle with redwater-gallsickness blood did not always result in a reaction to redwater. (No rise of temperature occurred and no *P. bigeminum* were noted in the smears. Animal remained apparently healthy).

"2. The blood of an animal which gave no reaction to redwater when inoculated with redwater-gallsickness blood does not transmit redwater to susceptible cattle.

"3. Susceptible animals which failed to react to redwater after inoculation are susceptible to redwater.

"4. The inoculation of a susceptible animal with blood which was collected from a beast when reacting to redwater, and in which *P. bigeminum* parasites were frequent does not always produce a redwater reaction (one experiment), and the animal remains susceptible to redwater.

"5. An animal which has reacted to a strain of redwater may again react when inoculated with blood of a different strain.

"6. The redwater reaction may manifest itself in a rise of temperature without the appearance of *P. bigeminum* in the smears during or shortly after the temperature reaction."

(2) Observations in Connection with the Transmission of Anaplasmosis.

(A) *The Invariability of Transmission of Anaplasmosis by means of inoculation of redwater-gallsickness blood.* From the above experiments the author also draws the following conclusions :—

"(1) That the inoculation of susceptible cattle with redwater-gallsickness blood did not always produce a reaction to redwater ; a reaction to anaplasmosis however invariably occurred.

"(2) The blood of animals which failed to react to redwater after inoculation with redwater-gallsickness blood produced in susceptible cattle anaplasmosis but not redwater.

"(3) Blood collected from an animal which had been inoculated with redwater-gallsickness blood during the redwater reaction and thus before the anaplasma infection appeared did not produce anaplasmosis in a susceptible animal.

"(4) The shortest period in which *Anaplasma marginale* appeared after inoculation was 20 days, longest period 44 days, average period 31 days. For *A. centrale* the shortest period was 16 days, longest 47, average 32 days."

(B) *The Mutability of Anaplasma centrale.*

Theiler has classified the anaplasmata into two varieties, viz., the *Anap. marginale* and *Anap. marginale* (variety *centrale*) ; the former is responsible for deaths occurring from genuine gallsickness contracted naturally while the latter is much less virulent and, when inoculated into a susceptible animal, transmits the Centrale infection and protects against the former (the *Anap. marginale* proper). [Hence Theiler's method of immunisation against gallsickness.] It was found that inoculation of the blood of an animal immune to the Centrale variety did not always result in a pure Centrale infection being transmitted, but produced in some cases a pure Marginale infection. A series of experiments was performed, which are recorded on the charts, in order to see whether mutation from Centrale to Marginale occurs in "passage" through animals. The author's conclusions are given in the following résumé :—

"The observations extended over a period of five years and a few days, namely, from 28th February, 1910, to the 8th March, 1915 ; during this time forty-three cattle were inoculated, and in thirty-five of these the experiment was controlled by the writer.

"*In the first series of inoculations* no mutation occurred during the passage through eight successive generations, viz. from the 28th February, 1910, to 30th December, 1913.

"An animal inoculated approximately eight months later than the latter date with blood of the seventh generation and two animals inoculated with blood of the eighth generation reacted to *Anaplasma marginale*.

"*In the second series of inoculations*, whereas no mutation occurred in some cases in passage through successive generations, viz. from the 28th February, 1910, to the 8th March, 1915, two cattle inoculated with blood of an eighth generation animal reacted to *Anaplasma marginale*.

"*In the third series of inoculations*, whereas centrale blood of the sixth generation produced a centrale infection, blood obtained from the same animal, approximately five and nine months later, produced a marginale infection and the inoculation of the blood of two imported animals which reacted to *Anap. centrale* resulted in a marginale infection.

"*Conclusion.*

"A mutation from *Anap. centrale* to *Anap. marginale* may occur in passage through a susceptible animal.

"The determination of the type is probably according to the Mendelian law, the marginale being the dominant—the centrale the recessive type."

(175) Veglia (F.). **The Cultivation of *Anaplasma marginale* in Vitro.**—*Union of South Africa. Dept. of Agric. 3rd & 4th Reports of the Director of Veterinary Research.* 1915. Nov. pp. 527–532. Pretoria: Govt. Printing & Stationery Office.

"The culture media utilised were :—

"(1) Pure defibrinated blood, viz., blood from an animal infected with anaplasmosis.

"(2) Salt solution (sodium chloride) as used by Nuttall for the cultivation of *Babesia canis*.

"(3) Sodium citrate solution, as used by Carpano for the cultivation of *Nuttalla equi*.

"(4) Ordinary bouillon, as used by Miyajima for the cultivation of *Babesia bovis*" . . . .

"*Conclusions* :—

"(1) Anaplasms grow and multiply in the different artificial media indicated above.

"(2) Defibrinated blood and Carpano media are most suitable for the cultivation of anaplasms.

"(3) Basophile granulations and Jolly's bodies, which are noted in anaemic blood, behaved differently to anaplasms on cultivation, inasmuch as they did not increase in number and finally disappeared.

"(4) Normal blood of cattle and other animals not infected with anaplasms did not develop any intracellular bodies resembling anaplasms when cultivated in the way indicated.

"(5) In cultures made with pure anaplasms or anaplasms and babesia mixed, no forms could be traced which would suggest a transition of one to another.

"(6) The defibrinated blood of a susceptible normal animal mixed with a culture appeared to be favourable for the development of the anaplasms, whilst the blood of an animal recovered from the recent attack of anaplasmosis appeared to inhibit the growth of the anaplasm.

"(7) The cultivation of anaplasms by a sub-culture has so far succeeded in the one instance attempted.

"(8) It appears that it is possible to grow anaplasms taken from an infected animal during the incubation stage of the disease.

"(9) The increase of anaplasms in the cultures corresponds to the increase of the anaplasms in the living animal.

"The oscillating decrease in the number of anaplasms noted in an animal that is recovering has its simile in the cultures."

### (c) Leishmaniasis.

(176) Finzi (Guido). **Leishmaniose et tuberculose chez le chien.** [Leishmaniasis and Tuberculosis in the Dog.]—*Bull. Soc. Path. Exot.* 1916. July. Vol. 9. No. 7. pp. 429–432.

Finzi records the case of a dog which had been brought for treatment to the clinic of the Veterinary School at Turin, and which later died of generalised tuberculosis. On examining preparations from the blood and spleen *Leishmania infantum* was easily discoverable. This dog had been brought from Cyrenaica a month prior to its death.

(d) Avian Malaria.

(177) Gonder (R.). **On the Transmission of *Haemoproteus columbae*.—*Union of South Africa. Dept. of Agric. 3rd & 4th Reports of the Director of Veterinary Research.*** 1915. Nov. pp. 625–632. Pretoria : Govt. Printing & Stationery Office.

Gonder expressed his views upon the natural transmission of *H. columbae* at the International Medical Congress in London in 1913, and the investigations upon which these views were based are published in this article. The following is the author's summary :—

"The infectivity of the parasite *Haemoproteus columbae* is not hereditary in the fly-carrier. The parasite develops in the fly, *Lynchia capensis (olfersia)*, only to the ookinete, which, through a double protoplasmic segmentation, loses all its pigment and a part of its protoplasm. So long as the ookinetes are present in the stomach of the fly transmission of the parasite by the fly is possible.

"The flies 'clean' themselves from an infection whenever they engorge on the blood of a healthy pigeon, but do not become immune against reinfection.

"A cleansing of the flies cannot take place so long as they feed upon infected pigeons.

"Artificial transmission from infected pigeons to healthy pigeons cannot be brought about by ordinary blood inoculation, but is easily effected by the injection of ookinetes cultivated in the moist chamber.

"Direct transmission is also possible if lung material be used."

(e) Sarcosporidiosis.

(178) Galli-Valerio (B.) **Are Sarcosporidia Aberrant Forms of Cnidosporidia of Invertebrates ?—*Jl. Parasit.*** 1916. Mar. Vol. 2. No. 3. pp. 126–128.

Darling (1915) suggests that, on account of the facility with which herbivora can ingest the bodies or the excreta of insects, etc., infected with neosporidia, it is possible that sarcosporidia may be "side-tracked varieties" of some of these parasites of invertebrates which are unable to continue further their life cycle in the muscles of an indefinite host.

Scott (1915) also infers from his experiments that the sheep is not the definite host of *S. tenella*.

The author states that it is probable that sarcosporidia are identical with cnidosporidia, and believes that if it is possible to demonstrate that spores of sarcosporidia produce amoebae the identity of the two orders is still more probable.

Piana (1896) obtained from cultures of *S. tenella* on certain media, bodies which presented motile amoeboid movements and later became encysted. The author himself (1913) confirmed these experiments with *S. muris*. It was not possible to produce Balbiana cysts in the oesophagus of a sheep infected with Piana's cultures.

Erdmann (1914) observed amoeboid bodies in the intestinal cells of a mouse infected *per os* with sarcosporidia.

Reference is also made to the similarity between the amoebae of certain myxosporidia and those of *S. muris*.

Thélohan and Auerbach proved that the spores of cnidosporidia produced amoeboid bodies in the duodenum of fishes.

The author believes that the amoebae obtained from cultures of sarcosporidia may be destroyed in the stomach and suggests that in future experiments they might be introduced direct into the intestines of sheep.

The author's summary is as follows :—

"(1) The observations of Piana and Galli-Valerio to the effect that spores of Sarcosporidia produce amoebic bodies in cultures, more closely relate the Sarcosporidia to the Cnidosporidia.

"(2) If true that Sarcosporidia are only aberrant forms of Neosporidia of invertebrates, then the hypothesis of Darling becomes more probable."

(179) Franco (E. E.) & Borges (I.). **Sur la sarcosporidiose bovine.** [Bovine Sarcosporidiosis.]—*Arq. do Inst. Bact. Camara Pestana.* 1916. Vol. 4. No. 3. pp. 269–289. With 10 coloured plates comprising 19 figs., & 1 plate comprising 4 figs.

In the course of the years 1885 to 1914, 67 carcases of cattle were rejected at the Lisbon Municipal Abattoir on account of sarcosporidiosis, representing 0·0563 per cent. of animals slaughtered. The animals affected were in the great majority of cases brought in from the Alentejo province.

The lesions affected the connective tissue, subcutaneous and intermuscular, and took the form of small cysts from 250 to 400 microns in diameter which resembled fine grains of sand in appearance. They were sometimes gathered together in groups, and sometimes disseminated at distances less than 5 millimetres apart. Lesions of "anasarca and elephantiasis," such as described by Besnoit and Robin, were not seen by the authors.

The article is almost entirely devoted to an analysis of the histology of the lesions ; the life history of the parasite is not studied.

*Cysts.*—These are composed of three parts, which are derived from the parasite itself, viz. :—(A) an external "anhist" membrane, (B) a cellular internal membrane consisting of a finely reticulated protoplasmic layer, and (C) the spore contents consisting of an enormous number of very small, closely packed, crescentic bodies.

Around the cysts is seen a zone of reaction of variable thickness consisting of (a) connective tissue cells, (b) eosinophile leucocytes, and (c) plasma cells.

In one of the cases histologically examined an account of cysts invading the dermis and the muscles is given.

A large number of the cysts were found to have become ruptured and, instead of spores, contain various connective tissue cellular elements.

The histology of the lesions is well depicted in the coloured plates.

The authors believe that the sarcosporidiosis observed by them is the same as that studied and described by Besnoit and Robin, due to the genus *Sarcocystis besnoiti* (Marotel, 1912).

### (f) Spirillosis.

(180) Blier (Jules). **L'hémoglobinurie bovine du Chili (Maladie à parasites spirochétiformes).** [Bovine Haemoglobinuria in Chili (a Disease Due to Spirochaetoid Parasites).]—*C. R. Acad. Sci.* 1914. Dec. 14. p. 815. [Extracted in *Rev. Gén. de Méd. Vét.* 1916. June 15. Vol. 25. No. 294. pp. 262–264.]

There exists in Chili, in the district of Santiago, a redwater of cattle commonly known as *Meada de Sangre.* It is a rather rare seasonal affection most frequent in autumn (i.e., February and March).

The characteristic symptoms are a discharge of blood-tinged urine accompanied by fever, exophthalmia, mania, vertigo, general icterus, and a discharge of blood from the anus, and the gums often bleed.

The disease generally runs its course in about 48 hours and always ends by the death of the animal. In farms where imported cattle are numerous, up to 5 per cent. of the bovines may succumb annually to the disease. However, throughout Chili it is a good deal less common than anthrax, with which it is often mistaken, and this confusion is aggravated by the presence of blackquarter. It had previously been noticed that the anthrax bacillus was absent from the spleen in these cases.

If the animal is slaughtered at the onset of symptoms the lesions show a considerable resemblance to those of yellow fever, viz., yellowish discolouration of the flesh and serous membranes, blood in the stomachs, hypertrophy of the gall bladder, haemoglobinuria, and liver affected with fatty degeneration and spotted with small haemorrhagic centres about the size of peas in which the parasite which is the cause of the disease can be found. Also, one notices hypertrophy of the spleen, profound changes in the suprarenal capsules, subcutaneous haemorrhages, often on the diaphragm a veritable roseola, and sometimes a blood-stained subcutaneous oedema. These lesions disappear if the disease is allowed to terminate with the death of the animal.

The clue to the cause of the disease is given by early slaughter. The specific germ was not perceived for a long time for, as in Sakharoff's spirillosis of geese, bacteriolysis takes place before death. In spite of the redwater the disease is not a piroplasmosis, and various observers have confirmed this point (Lucet, Lignieres, Sivori, Mamerto Cadiz, Mabilais, Blier). Ixodes have never been found on affected farms.

The redwater is caused by an organism which the author considers he discovered in 1912, and which he has always been able to discover since then on slaughter at the onset of symptoms. The organism apparently is not easy to classify. It recalls at first sight a spirillum or spirochaete, but it differs from them in that it possesses a plasticity which enables it to execute contractile movements. It is longer as a rule than the majority of known spirochaetes, and it may exceed 60 microns in length and its breadth then is about 1 micron. The shorter forms are swollen towards the centre. It is sometimes provided with a few undulations of regular length but these are never found in the same part of the body. One extremity of the organism is much thinner than the other. It stains uniformly with Giemsa. When stained with Borrel's blue and eosin it sometimes shows bright red, refractile granules. Ordinarily the organism has the form of a spirochaete, but it recalls the parasite described by the brothers Sergent (genus *Sergentella* of Brumpt, 1910) and other observers.

The author considers that it is the causal organism of the disease because when taken from the small haemorrhagic lesions in the liver of a subject slaughtered at the commencement of symptoms, desensitised by washing for 15 minutes in lukewarm sterile broth, and inoculated into the abdominal cavity of cattle it reproduces the disease, but with mild symptoms (slight fever, hepatic roseola, biliary haemoglobinuria, and the presence of the parasite in the lesions).

The period of incubation is from five to six days and during this period one can recover the microorganism from the blood by means of centrifugalisation. As in the case of yellow fever the disease can only be transmitted at the onset of symptoms. The desensitisation of the virus appears indispensable and all other methods of inoculation have failed in the author's hands.

The specific organism was not found in numerous post-mortem examinations of animals not affected with the disease, and in the affected animals it can only be found in the lesions, where it sometimes requires considerable search. It has never yet been seen alive.

As in the case of yellow fever recoveries occur; relapses may take place, but never in the same season, and they are exceptional. It is probable that they are due to re-infection. As they take place at intervals of several years a certain degree of immunity is probable. Young animals are never infected. A single case of transmission to the foetus has been observed by the author.

The sporadic nature of the redwater strongly points to infection by indirect contagion. The transmitting agent is unknown but many breeders incriminate an acarus of the genus *Tetranychus* which lives in the grass and weaves webs and is ingested by cattle whilst grazing. This opinion is very much disputed, but the disease is sometimes known under the name of *tela araña* (spiders web). Some farmers get rid of this web by harrowing the pastures. The heavy autumnal rains cause the enzootic very nearly to disappear.

It seems that one or more substances of unknown nature considerably delay putrefaction in animals dead of the disease.

(181) Velu. **Sur la spirillose equine au Maroc.** [Equine Spirillosis in Morocco.]—*Rec. Méd. Vét.* 1916. Apr. 15. Vol. 92. No. 7. pp. 215–224. With 2 text-figs.

Equine spirillosis has been described by Theiler in the Transvaal, by G. Martin in French Guinea, S. Dodd in the Transvaal, Stordy in British East Africa, and Carpano (Matteo) in Erythrea.

The specificity of *Spirillum equi* has not yet been established. Experimental inoculations seem to have been difficult to realise. The spirillary infection is without doubt often associated with other affections such as trypanosomiasis or horse sickness. Also the differences between the pathogenicity of the various equine spirilla encountered are very considerable.

Velu has studied the disease in three horses naturally infected, which were kept under his observation. Clinically the disease presented the same aspect as that of trypanosomiasis affecting orses in Morocco, viz., a mild fever accompanied with oedema of the sheath, etc., from which the animals apparently recovered after a couple of months. Examination of the blood during the course of the symptoms only on very few occasions showed some rare spirilla.

Velu succeeded in transmitting the disease by inoculation to white rats, dogs, a rabbit and two fowls, and he thus summarises his results: "Spirillosis of the horse is transmissible to the dog, rabbit, and rat. Animals which receive subcutaneously or intravenously from 3 to 20 cc. of horse blood which does not disclose any visible spirilla under the

microscope, become affected with a disease which terminates fatally and is characterised by low fever, progressive emaciation, and numerous parasites in the peripheral blood. The spirilla disappear pretty quickly from the general circulation. It is possible to transmit this spirillosis by ' passage ' from the dog to the rabbit and the rat."

The spirilla measured from 12 to 21·6 microns in length when not uncoiled, by from 0·25 to 0·3 microns at most in width, and contain from four to six spiral turns. Forms about to undergo transverse division may attain a maximum length of 33·6 microns and present up to 10, or even 12, spiral turns. The spirillum stains uniformly throughout its body.

(182) Robert (A. Eug.) & Sauton (B.). **Action du bismuth sur la spirillose des poules.** [The Action of Bismuth on the Spirillosis of Fowls.]—*Ann. Inst. Pasteur.* 1916. June. Vol. 30. No. 6. pp. 261–271.

The authors utilised for their experiments sodium bismuthotartrate, as aqueous solutions of this compound can be sterilised in the autoclave and they are stable in the presence of the majority of salts, serum, etc., but are, however, precipitated by calcium salts. In all their experiments they used solutions containing exactly one per cent. of bismuth itself, not of the bismuthotartrate.

*Toxicity.* A dose of from 30 to 35 mg. of Bismuth per kilog. body weight injected intravenously can be tolerated by a healthy fowl; animals infected with spirilla can only stand a dose of 15 to 20 mg.; 100 mg. of bismuth per kilog. injected intramuscularly can be tolerated without any inconvenience by healthy fowls.

*Action in vitro.* When equal parts of blood from a fowl severely infected with spirilla and a neutral solution of sodium bismuthatartrate containing 1 per cent. of bismuth are mixed, *Spirillum gallinarum* retains its motility for more than three days.

*Action in vivo.* The authors used as virus undiluted blood rich in spirilla.

*Preventive Treatment.* The intravenous injection of 20 to 30 mg. of bismuth per kilog. 24 hours before inoculation with 0·5 cc. of infective blood delays the appearance of spirilla by a day and then the disease develops normally, probably due to a too rapid elimination of the salt or to its having become partially insoluble in the body. On the other hand in the same conditions of time an intramuscular injection of 50 to 70 mg. of bismuth per kilog. acts efficaciously.

The injection of bismuth a few hours after infection always retards the development of the organisms. At no period can spirilla be found in the blood of a fowl injected intravenously six hours after infection with a dose which, however, is not well tolerated—of 20 mg. of bismuth per kilog. In the same conditions an injection of 15 mg. does not always prevent the disease to the same degree. The disease when it manifests itself is, however, benign and always disappears after a second injection. Treatment with 10 mg. brings about only a slight delay in the appearance of spirilla and the disease then develops as in control animals.

Better results were obtained by giving two successive injections of bismuthotartrate, 6 hours and 30 hours respectively, after inoculation with spirilla. The organisms never appeared in the blood of animals treated with two doses of 15 mg. each. Positive results were also obtained in four cases out of every five by two injections of 10 mg. each, or by successive injections of 10 and 5 mg., respectively. It is noteworthy that these two last doses are more efficacious than a single equivalent dose of 15 mg. because the circulation of bismuth in the body is renewed.

An intramuscular injection of 60 to 70 mg. of bismuth per kilog. four hours after infection delays and considerably alleviates the disease. Spirilla only appear in the blood of animals thus treated in very small numbers and during a very short period of time.

*Curative Treatment.* When, after the first appearance of the spirilla, one treats an infected animal by giving an intravenous injection of 12 to 15 mg. of bismuth per kilog. the spirillosis takes on a milder course, and fowls thus treated generally show organisms no longer in their blood when the infection is at its maximum in the case of control animals. If intervention takes place later, 48 to 72 hours after infection, a distinctly curative action of the bismuth is observed and the disease never takes on an acute form, which in the authors' experiments caused the death of 33 per cent. of animals which were not treated.

However, the best results are obtained by treating infected animals with two successive intravenous injections of 10 mg. per kilog., 30 and 48 hours, respectively, after inoculation with spirilla. The organisms then never, or hardly ever, develop in the blood after the first injection; they have always disappeared 24 hours after the second. The treated animals remain in good condition and do not as in the case of control animals lose a considerable amount of weight.

Conclusions. "From these experiments it results that sodium bismuthotartrate, contrary to what one observes *in vitro*, exerts a bactericidal action on *Spirochaeta gallinarum* in the animal body. Its influence, which was very distinct since we were able to prevent and cure the infection in a relatively large number of cases, was not, however, always manifested in our experiments with all the regularity desirable. This depends probably on the nature of the bismuthic combination utilised, which, from our observations, may lose its activity as a result of fixation or insolubilisation. It is presumable, and our experiments, abruptly interrupted at the end of July 1914 by the war, already confirmed this hypothesis, that the best results would be obtained by the use of bismuthic compounds susceptible of circulating better in a soluble form in the body. We hope to continue some day our incomplete researches in that direction, but we can already say that we have observed the constant efficacy of bismuth, whatever may be the nature of the chemical combination it forms part of."

## (g) Toxoplasmosis.

(183) Carini (A.) & Migliano (L.). **Sur un Toxoplasme du cobaye (*Toxoplasma Caviae*, n. sp.).** [A Toxoplasm of the Guinea-pig). —*Bull. Soc. Path. Exot.* 1916. July. Vol. 9. No. 7. pp. 435–436.

In a young guinea-pig found dead without having shown previous symptoms the authors discovered the presence of toxoplasms.

On post-mortem examination the spleen was three or four times the normal size, friable, and soft in consistence; the liver was increased

in size and congested, the lungs showed some congested centres, and the intestines also were reddened.

Microscopic examination of the affected organs showed, after staining with Leishman or Giemsa, bodies which presented all the characters of toxoplasms. They were 5 to 8 microns long by 2 to 4 microns broad, generally a little curved, oval or pear-shaped, rarely rounded or irregular. The protoplasm stained a pale blue colour and the nucleus a reddish-violet. The parasites were generally free, but sometimes endocellular. In preparations from the liver, organisms undergoing multiplication were found in the form of masses of protoplasm in which were scattered numerous nuclei.

Two pigeons were inoculated subcutaneously with an emulsion of the affected organs. They both died in 17 days. For the first 14 days no visible symptoms were observed; during the last three days there were manifested an intense thirst, inappetence, and during the last day the pigeons became very dejected and their feathers stood erect.

On post-mortem examination the following lesions were noted, viz., on the surface of the lungs numerous small greyish spots which corresponded with centres of necrosis, and the liver was very much enlarged and congested. The parasites were very numerous in the liver, and still more numerous in the lungs.

The authors state that they had previously observed the presence of toxoplasms in guinea-pigs, but these had been inoculated with various pathological products, and so they were not certain that they were dealing with spontaneous infections. Several observers have already noted that guinea-pigs can be experimentally infected with toxoplasms.

The authors believe that the case recorded by them enables them to state definitely that one may encounter spontaneous infections with toxoplasms in the guinea-pig. It is probable that the toxoplasm seen in the guinea-pig is the same as that of the rabbit and the dog, but this has not yet been proved, and the name *Toxoplasma caviae* is thus suggested.

The following list of toxoplasms recorded up to the present is given :–

| | | |
|---|---|---|
| *Toxoplasma cuniculi* | .. | Splendore, 1908. |
| ,, *gondii* | .. | Nicolle and Manceaux, 1908. |
| ,, *canis* .. | .. | Mello, 1910. |
| ,, *talpae* | .. | Mine, 1910. |
| ,, *columbae* | .. | Carini, 1911. |
| ,, *musculi* | .. | Sangiorgi, 1913. |
| ,, *avium* | .. | Adie 1909; Marullaz, 1913. |
| ,, *pyrogenes* | .. | Castellani, 1914. |
| ,, *ratti* .. | .. | Sangiorgi, 1914. |
| ,, *caviae* | .. | Carini and Migliano, 1916. |

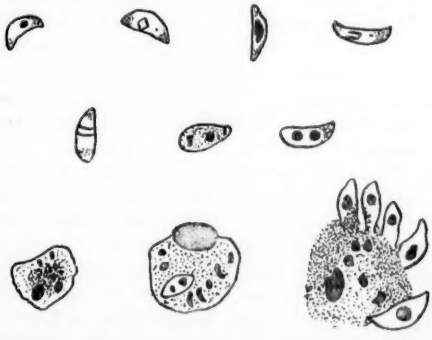
(184) van Saceghem (R.). **Observations sur des infections naturelles par *Toxoplasma cuniculi*.** [Observations on Natural Infections by *Toxoplasma cuniculi*.]—*Bull. Soc. Path. Exot.* 1916. July. Vol. 9. No. 7. pp. 432–434. With 10 text-figs.

Natural cases of the above disease were found by the author in rabbits born in the Lower Congo; the disease was only observed in the case of young subjects from 2½ to 3 months old.

The rabbits which became affected were kept in a large box supported by four poles about 27 cm. high. Rabbits in cages placed at the height of 1 metre remained all unaffected. The author believes that this fact suggests that, if an insect is the transmitting agent of *Tox. cuniculi*, then this insect can only move about at relatively low heights and that it is probably wingless.

The symptoms are those of a profuse anaemia and inappetence, followed later by paralysis just before death. On post-mortem examination one finds a yellowish exudate in the peritoneal cavity, very considerable enlargement of the spleen, which is covered with whitish spots and ecchymosed, and the liver as well as the lungs are covered with similar spots, which, at first sight closely resemble miliary tubercules. The blood shows basophilia and lymphocytosis, but no parasite was found in the peripheral circulation.

Smears from the spleen stained with Giemsa showed up quite easily the typical parasite of SPLENDORE. Beside the characteristic crescentic forms the author discovered more or less spherical organisms. The average length of the parasite was from 6 to 7 microns although certain forms undergoing division attained 21 microns in length. The position of the nucleus is very irregular; it may be central, terminal, or in some intermediate position. One or two chromatin granules were sometimes found within it.



The parasite is found in the large mononuclear cells of the spleen, and in some of these cells it may be seen undergoing a process of division by schizogony. At a certain stage the large mononuclear cell, whose nucleus has been pushed towards the circumference, becomes broken up, and young merozoites are set free (see the two figs.* on right of lower line). Forms up to 21 microns in diameter were seen, more or less circular in outline, and containing a large number of nuclei. The author could not find the remains of the nucleus of the large mononuclear cell in them and so inferred that these large forms of the protozoon are in reality schizonts. The protoplasm of the parasite is very irregularly stained by Giemsa; in some cases it stains an intense blue, in other cases it does not stain at all.

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## PARASITOLOGY.

### (a) Helminthiasis.

(185) Descazeaux (M. J.). **Contribution à l'étude de l' "esponja" ou plaies d'été des Equidés du Brésil.** ["Esponja" or "Summer Sores" of Equines in Brazil. (Railliet's Report of a Commission to the *Soc. Cent. de Méd. Vét.* 17 June 1915).]—*Rec. de Méd. Vét.* 1915. Jan. 30 to Sept. 30. Vol. 91. Nos. 19–20. pp. 468–486. With 3 text-figs.

In this report Railliet analyses the whole question of "summer sores" of equines, basing his conclusions chiefly on information collected by Descazeaux, who has studied the disease in Brazil.

A very clear and concise description is first given of the clinical characters and morbid anatomy of the disease, particularly as it occurs in Brazil. The granular dermatitis is stated to present numerous variations in its characters according to the countries in which it is observed, as for example in India, Africa, Oceania, and in tropical America.

The report is mainly of importance in that it throws new light on the etiology of summer sores. It is well known that in numerous observations the presence of a nematode has been determined in the granulations of "summer sores," but our clinical knowledge is not yet so precise that one can differentiate, by means of a simple external examination, between verminous dermatitis and dermatites attributable to other elements than worms. It is thus that in India one still often confuses, under the name of *bursati* or *bursattee*, cutaneous lesions caused by moulds and by nematodes.

Ercolani was the first to discover, in 1859, a larval nematode in the loose fibrous tissue at the base of the newly formed "umbilicate scabs" and he remarked that this larva bore a good deal of resemblance to the embryos of *Spiroptera megastoma* (now called *Habronema megastoma*). More detailed descriptions have lately been given of this parasite by Buffard, Rivolta, and Fayet and Moreau.

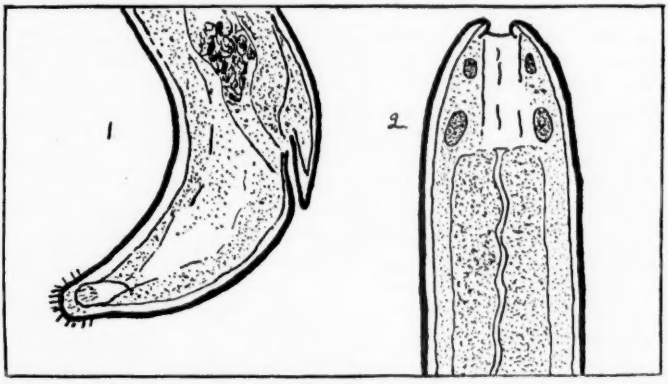
Descazeaux obtained the nematodes from granulations about 15 to 20 days after their first appearance. A piece of non-calcified granulating tissue was thus placed on a slide with a drop of 0·2 per cent. hydrochloric acid. After about five minutes the tissue is broken up by means of coarse dissection with a pair of fine needles. One looks under the microscope in the dissociated pieces for the presence of the nematode and a head or a tail is found pretty easily. One then retains only the small piece of tissue containing the parasite, keeping a watch on its dissolution and helping it by means of cautious dilaceration, for the worm is easily broken. As soon as the latter is isolated the slide is dried and the specimen covered with a cover glass. A drop or two of lactophenol is introduced between the slide and cover glass, and the latter is then bordered with paraffin wax.

Descazeaux describes two kinds of parasite, viz. :—

(A) *Constant parasite.*—"Asexual" nematode, 2 mm. long by 45 to 50 microns broad. Cylindrical, filiform, slightly flattened body, thinner at its anterior extremity and terminating posteriorly in a blunt end covered with bristles; elongated intestine; flexuous uterus which "seems" to contain spherical eggs from 5 to 6 microns in diameter.

(B) *Inconstant parasite.*—This was discovered in one sore only out of more than ten examined, and was described as an "asexual worm" 900 to 950 microns long by 25 microns broad; elongated, cylindrical body, slightly thinner in front and terminating in a sharp drawn-out point. Said to contain a bifurcated uterus full of elongated eggs.

Railliet maintains that Descazeaux has here committed the same mistake as other authors, such as FAYET and MOREAU, in that he mistook the cells of the intestinal wall for eggs. One knows of no nematode possessing eggs of from 5 to 6 microns in diameter, so that the alleged uteri which he describes are, in reality, inexistent.




**Larva A.—1, posterior extremity showing end covered with tuft of bristles, and above and to the right, the anus; 2, anterior extremity showing mouth, vestibule or pharynx, and oesophagus with tortuous lumen.***

Railliet considers that the presence of the tuft of bristles at the extremity of the tail in the larval form (A), corresponding with what RIVOLTA had also previously described at the caudal extremity of his parasite, is of fundamental importance in that it enables one to determine approximately the classification of the nematode of "summer sores." Larvae with bristly tails have already been observed in a large number of animals—insects, etc.—and have been lately given a new name—*Agamospirura*—by HENRY and SISSOFF to indicate their origin. They represent in reality the larvae of nematodes commonly designated under the name of *Spiroptera* (suborder *Spirurata* or super-family *Spiruroidea*).

In searching for the adult form of the larva of "summer sores," one can thus limit the field to the only genus of spiruroidea of which we know representatives in the horse, viz., the genus *Habronema* (Dies). The three species of spiroptera parasitic in equines belong to this genus, viz., *Habronema megastoma* (Rud.), *H. microstoma* (Schneid.), and *H. muscae* (Carter).

The evolution of the last named of these species has been described by RANSOM, and in his report Railliet summarises this evolution. As far back as 1861 CARTER, had found a larval nematode in the proboscis, head, and abdomen of the domestic fly. RANSOM showed that this larva

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belonged to a spiroptera, *H. muscae*, which lives in the stomach of the horse; the embryos evacuated with the faeces penetrate into the larvae of flies (living on horse manure), undergo development in these larvae, as well as in the pupae and fully formed insects, and finally reach the adult state in the stomach of the horse, when the flies are ingested by this animal. Besides the initial embryo and free-living form, RANSOM was able to distinguish six stages in the development of the larva within the body of the fly.

These stages, separated from one another by a moult, took place, the first two in the pupae or larvae, and the others in the winged fly. The nematode larvae increase in size from 400 to 450 microns in the first stage up to 2 mm. in length in the sixth or last stage. The bristles appear at the end of the tail in the fifth stage. The last stage of evolution in the fly is the first in the horse's stomach, where RANSOM collected it from among other forms more advanced in development and even adults. After about four or five moults in the stomach the worm reaches its adult state; the tail bristles are present after the first stage in the stomach but disappear afterwards.

The method of infection in the horse has not been accurately determined, but perhaps the larva escapes from the fly's proboscis while it feeds on the moist lips of the animal, or perhaps the horse also is infected by swallowing living or dead flies. The various forms of nematode described by different observers would correspond with the embryos or one or other of the larval stages of *Habronema*.

As regards the method of penetration of these larvae through the horse's skin, one is as yet limited to hypotheses. RIVOLTA believed that the penetration was from without inwards, and that it was favoured by the dirty condition of the animals and of stables. Descazeaux shares this view because he noticed that the "esponjas" are confined for the most part to the lower part of the limbs, and that certain ordinary wounds may at the end of a certain time become transformed into "esponjas." On the other hand LAULANIÉ noticed the arterioles of the dermis in course of obliteration and thus thought that the parasite was conveyed in the circulating blood. Also, LINGARD in India, whilst studying "*Bursati*" which he attributed to embryos circulating in the blood, pointed out that the presence of the characteristic granulations of these lesions was observed before the formation of an ulcer. One knows, however, that the larvae of Ankylostoma and Strongyloidae are able to penetrate through the skin of mammals without leaving any apparent traces of their passage.

Railliet is inclined to think that the nematodes penetrate from without inwards in the following manner. The *Habronema* embryos are thrown into the outside world with the excreta of the horse; they then penetrate through the skin of the animal in the same way as the larvae of ankylostoma, there setting up an irritation which may lead, by rubbing or scraping, etc., to the formation of a sore which takes on from presence of these larvae its particular characteristics. They develop as erratic parasites in this abnormal medium and undergo in it an evolution analogous to that which they normally accomplish in the body of the fly.

*Treatment.* Up to the present the *Spiroptera* have been looked upon as almost inoffensive parasites of the horse's stomach and no attempts are usually made to get rid of them. One knows now,

however, that the nematode of "summer sores" belongs to the cycle of evolution of one or several kinds of these parasites. Thus *a priori* the solution of the problem lies in the expulsion of these parasites from the horse's stomach, and one might try experiments in the use of tartar emetic or arsenious acid for this purpose.

Various medicaments employed locally have been of little or no use in the treatment of the disease, except that Descazeaux states that he obtained good results by rubbing the sores with 2 to 3 per cent. solutions of trypanblue. The only effective treatment is, however, the early and complete excision of the infected tissues.

(184) Migone (L. E.). **Parasitologie de certains animaux du Paraguay.** [Parasitology of Certain Animals of Paraguay.]—*Bull. Soc. Path. Exot.* 1916. June. Vol. 9. No. 6. pp. 359–364.

The chief parasites are *Haemogregarinae*, *Trypanosoma*, *Microfilaria*, *Myxosporidia*, and a parasite not well understood at present, *Linguatula*.

The author gives a list of birds, fishes, reptiles, and mammals with short descriptions of the parasites found in the blood and tissues of each. From his observations he deduces that there exist, in South America, parasites identical with those already known in the old world and in the other countries of America.

*Linguatulae.* When one finds a fish affected, one observes at first sight in the abdominal cavity numerous linguatulae and cysts of small linguatulae. The peritoneum, mesentery, and parietal peritoneum are all affected with these parasites. This arthopod penetrates through the intestinal canal, its head always being directed towards the peritoneal cavity. Alongside the well-developed parasites from 1 to 3 centimetres long there are numerous cysts 1 to 3 mm. in diameter containing a little liquid and a small worm completely formed with its pseudo-articulations and hooks. The author believes that the parasite is *Linguatula serrata*. The parasite is also encountered in mammals which feed on the dead fish commonly found on the borders of streams and dried up lakes.

(b) Acariasis.

(185) Shilston (A. W.). **Sheep Scab. Observations on the Life-History of *Psoroptes communis* Var. *ovis*, and some Points connected with the Epizootiology of the Disease in South Africa.** —*Union of South Africa. Dept. of Agric. 3rd & 4th Reports of the Director of Veterinary Research.* 1915. Nov. pp. 69–98. Pretoria: Govt. Printing & Stationery Office.

Practically the whole of our knowledge of the life-history of the acarus of sheep scab has been derived from investigators who recorded their observations over 50 years ago, notably Gerlach (1857). The observations of Stockman were published while the experiments recorded by the author were in progress.

Very little attempt has been made to find out whether the life cycle of the sheep scab acarus differs in countries with widely different climatic conditions. Hence, a series of extremely interesting experiments were carried out by Shilston in which the complete life cycle of the parasite in South Africa was worked out.

In order to watch the development of the psoropt in its various phases on sheep the following technique is employed. The small patch of skin on which the parasites are to be deposited is carefully washed and shaved, and a glass cylinder three inches high by two inches in diameter is placed on it and secured around its base by means of melted wax. Its open end is tied over with a piece of muslin to prevent escape of the parasites; the adjoining locks of wool are then securely tied over the cylinder to prevent slipping. The parasites were moved on to a fresh spot every three or four days.

*Life History on Sheep.*—The weather has very little effect on the duration of the life cycle. It never occupies more than 10 days and in the great majority of cases it is complete in 9 days. The eggs usually hatch out after an interval of two days. The larval stage is short, nymphae usually appear 48 hours after hatching of the eggs and always within three days. The nymphal stage lasts from three to four days. Pubescent females always appear before males. The former were observed 5½ days after the hatching of the eggs but the latter were never seen earlier than the sixth day. The females are three or sometimes four times as numerous as the males. After feeding for a short time copulation takes place which lasts for about 24 hours. After moulting the ovigerous female is then formed, and it may commence to lay eggs 24 hours after this last moulting, that is, nine days from the time of hatching of the larva from the egg.

It is rather difficult to find out the number of eggs which can be laid by a female. In a number of observations it was found that a single female is capable of laying up to 100 eggs, but that the number laid depends largely on weather conditions. Laying usually commences on the 9th day and may continue up to the 38th day. The life of the ovigerous female on the sheep is from 30 to 40 days.

The longest period over which an unfertilised female was kept alive on a sheep was seven weeks, though the majority of such parasites died three or four weeks after moulting from nymphae. Male acari lived for periods varying from 22 to 34 days after moulting from nymphae.

*Factors in the Epizootiology of Sheep Scab bearing on the Question of Eradication.*—A single dipping is known to fail to cure scab owing to the escape of the eggs. The life-cycle being complete in nine days, dipping should thus be repeated in 10 days at the most. An extra day may be allowed because the fleece usually remains wet for a day after the first dipping. Owing to the protection of crusts, even adult parasites may be allowed to survive two immersions, hence a third dipping ten or fourteen days after the second would greatly increase the chance of a complete cure.

A number of observations were made to ascertain the length of time acari and their eggs can retain their vitality apart from sheep. In crusts and wool kept at room temperature the acari begin to die in three or four days, the young forms first, and are generally all dead in 16 days after removal from the sheep.

When kept moist at body temperature (37° C.) in an incubator the acari are very active for two or three days and eggs present in the crusts hatch. A day or two later the larvae and most of the older forms die and very few survive more than six or seven days.

At 0° C. all forms were dead in four days.

Eggs kept at air temperature for up to, and including, eight days after being laid, hatched out when placed on sheep; after nine days removal one egg hatched out of 20, and after longer periods not one hatched. In an ice box the eggs retained their vitality for 10 days, but after 12 days all failed to hatch. It is thus no longer possible to regard the eggs of acari as being capable of maintaining their vitality for long periods apart from sheep and thus accounting for the persistence of the disease in kraals, etc.

The following table gives the results of experiments undertaken to show the period over which infection with sheep scab parasites may persist :—

| Expt. No. | Particulars of Infection. | Interval between Removal of Infected Sheep from Kraal and Contact with Clean Sheep. | Length of time Clean Sheep remained in Contact. | Result. |
|---|---|---|---|---|
| 1 | 37 Scabby sheep herded in Kraal for 44 days | 21 days | 76 days | Sheep clean. |
| 2 | 30 Scabby sheep herded in kraal for 48 days | 60 days | 16 days | Sheep clean. |
| 3 | Do. | 90 days | 7 days | Sheep clean. |
| 4 | 37 Scabby sheep herded in kraal for 35 days | 10 days | 60 days | Sheep clean. |
| 5 | Infected sheep running in a kraal for several months | 24 days | 48 days | Sheep clean. |
| 6 | Wool crusts and acari spread in box | Box kept free of sheep for 20 days | 4 months | Sheep clean. |
| 7 | Acari and pieces of fleece scattered in box | Box kept free of sheep for 15 days | 4 months | Sheep clean. |
| 8 | Manure collected from a kraal in which infected sheep had been running up to 16 days previously | Clean sheep immediately put into :— | 5 days | Sheep infected. |
| | | (*a*) clean kraal with manure, | | |
| | | (*b*) above sheep removed after five days and replaced by second lot of clean sheep | 6 months | Sheep clean. |
| 9 | 2 Scabby sheep running in a pen for 7 weeks. At the time of removal, pieces of fleece and acari were spread on floor of pen | 8 days | 4 months | Sheep clean. |
| 10 | Do. | 12 days | 4 months | Sheep clean. |

From the above it can be seen that acari are unable to live for more than four weeks apart from sheep.

Investigations showed that the psoroptes causing scab and otacariasis in goats and rabbits could not cause scab in sheep. The converse was equally true.

*Variations in the Rapidity of Multiplication of the Acari.*—As in Great Britain, great variations in the prevalence of sheep scab in the course of the year are also observable in South Africa. On scabby sheep, maintained at the Laboratory to supply material for experiments, the number of acari was noted to diminish in the spring of each year until, later, they could only be found with considerable difficulty. It is also well known to sheep farmers in very dry areas that, in certain seasons of the year, flocks that have been badly infected may become apparently clean and remain so without any kind of treatment being carried out. When on account of drought the sheep lose condition scab again makes its appearance, although no opportunity for re-infection may have occurred in the meantime.

With sheep in good condition it is frequently found difficult or even impossible to transmit the disease by infection with acari. "In carrying out the observations on the life-history of acari it was frequently noticed that development occurred just as rapidly on the washed and shaven patches on affected sheep as on those in poor condition, but when the covers were removed and when the parasites were allowed to spread to the surrounding skin multiplication was much more rapid in the case of the thin sheep than on those with excess of yolk in their fleece, while in a few of the latter no development occurred at all."

In order to observe the *effect upon the acari of the presence of wool fat*, two patches were prepared on the same sheep; one was washed and shaved in the usual way, but on the other the wool was only cut short and in addition the patch was smeared with "yolk" obtained from the wool of another sheep. On each patch six ovigerous female acari were placed. It was seen that in both cases eggs were laid on the following day and larvae were present on the fourth day.

On the ungreased patch the development occurred as usual, eggs being laid nine days after the appearance of the larvae, and this cycle was continued for several generations. As each generation of acari began to develop on the ungreased patch, a number of the young forms was removed to a fresh area so that the number of generations could be determined.

On the greased patch, however, the majority of the larvae died two or three days after hatching, while those that survived developed very slowly; thus it was not till 14 days after hatching that adult male and female acari were observed, and although fertilisation took place no eggs were laid, and living acari were not observed after the 21st day from the time of hatching. By this time the fourth generation of acari were developing on the ungreased area.

When the skin was greased very slightly the acari developed as rapidly as on the ungreased area. In these cases, the growing wool lifted the first crusts formed and these carried the fat with them so that the acari were able to live on the dry skin underneath. However, when sufficient grease was present to soak down under the crusts development was very slow and in every case the acari gradually died out, usually in about 20 days.

## MYCOLOGY.

### (a) Aspergillosis.

(188) Walker (J.). **Aspergillosis in the Ostrich Chick. (Synonyms: Yellow Liver, Chick Fever).**—*Union of South Africa. Dept. of Agric., 3rd & 4th Reports of the Director of Veterinary Research.* 1915. Nov. pp. 533–574. With 5 charts & 2 plates, comprising 7 figs. Pretoria: Govt. Printing & Stationery Office.

Chick Fever or Yellow Liver among ostrich chicks reared on some farms in South Africa sometimes causes a mortality as high as 70 per cent. The disease has thus assumed considerable economic importance, inasmuch as prices as high as £200 to £300 may be paid for adults, and £30 for three month-old chicks.

In preliminary observations the author discovered that various moulds, notably of the Aspergillus type, were found in the lesions of chick fever and, in exposure experiments, were capable of transmitting the disease.

On a plan outlined by the Director of Veterinary Research of the Union of South Africa, the author carried out further detailed investigations into the nature of the disease, the results of which are summarised in the following conclusions :—

" 1. *Aspergillus fumigatus* appears in the ostrich, more particularly in the ostrich chick, from a few days to about 3–4 weeks after hatching, and is responsible for the disease in ostrich chicks commonly known as 'Yellow Liver or Chick Fever.'

" 2. *Aspergillus fumigatus* is the commoner and more pathogenic species.

" 3. Outbreaks usually appear in an epidemic form and are more prevalent amongst chicks artificially hatched and reared.

" 4. *Aspergillus fumigatus* infection occurs in the air chamber of the egg, and is common in straw and other vegetable matter and in soil which has been fertilized with decomposed vegetable matter, such as stable manure, etc.

" 5. Infected eggs are the chief source of infection of incubators, the liberation of *Aspergillus fumigatus* from the air-chamber taking place either at the time of hatching or when infected eggs are opened in the incubators.

" 6. Aspergillosis is contracted naturally from :—

" (*a*) Infected eggs just previous to or at time of hatching.

" (*b*) Infected bedding used in chicks' sleeping boxes.

" (*c*) Infected incubators.

" 7. Aspergillosis may be transmitted artificially by inhalation and ingestion and intravenous inoculation of cultures.

" 8. Aspergillus infection occurs chiefly through the respiratory tract, the lungs and air-sacs being the seat of infection.

" 9. Infection may occur through the digestive tract.

" 10. *Aspergillus fumigatus* is transmitted from infected to clean eggs through the unbroken shell.

" 11. The contents of unbroken eggs may escape through the intact shell. In such cases the bacteria which exist in the contents are probably a source of infection of eggs, more particularly those in contact.

" 12. Spores of *Aspergillus fumigatus* vegetate after passing through the intestinal canal.

" Prevention consists in the use of :—

" (1) Non-infected incubators.

" (2) Non-infected bedding in the chicks' sleeping boxes.

" (3) Non-infected eggs for incubation purposes.

" Boiling water has given satisfactory results in the sterilization of cultures of *Aspergillus fumigatus*."

(b) Cryptococcosis.

(189) Monod (T.) & Velu. **Sur la lymphangite épizootique au Maroc.** [Epizootic Lymphangitis in Morocco.]—*Rec. de Méd. Vét.* 1915. Oct. Vol. 91. No. 18. pp. 631–638. With 4 figs.

(1) *Recurrent attacks of epizootic lymphangitis.*—The authors have never seen cases of re-infection, but on the other hand have observed local delayed relapses equivalent to re-infections. Four cases are detailed in which relapses occurred from three to six months after apparently complete recovery from a previous infection.

(2) *Osseous lesions in epizootic lymphangitis.*—Extra-cutaneous lesions are often secondary and the associated abscesses in those cases facilitate diagnosis, or they may be complications of the lymphangitis. In rarer cases these extra-cutaneous lesions may be the only cryptococcic localisations clinically discoverable.

Osseous cryptococcosis was found in 17 out of 159 cases treated in the course of a year. Hitherto, the cryptococcus was not suspected to be the causal agent of these lesions, but it appears in reality to exert a predilection for bony tissues.

Clinically one can distinguish (1) ostites associated with cutaneous lesions and (2) primary ostites.

A description of 17 cases, with photographs of four lesions, is given, in which lesions of localised and diffuse suppurative periostitis, periostoses, suppurative osteomyelitis, hypertrophying ostitis and spontaneous fracture are described, involving various bones such as the maxillae, scapula, sternum and the pelvis. These lesions simulated all the forms of chronic ostitis due to other causes, and might be easily confused with them. The evolution of these cryptococcic ostites is always chronic, and they are most often post-traumatic lesions.

Diagnosis is difficult and can only be settled by means of the microscope.

Prognosis.—Bony lesions constitute the most serious and most refractory of all the localisations. Out of 20 cases of epizootic lymphangitis slaughtered as incurable in a year, 13 had bony lesions.

Treatment should be the same as in cases of typical epizootic lymphangitis. Surgical intervention is only indicated in cases where a sequestrum is formed and good results are then obtained in three cases out of every four. The successful results hitherto obtained in these cases by excision caused one to overlook the fact that the lesions were in reality cryptococcic in origin.

(190) Bridré (J.). **La lymphangite épizootique en France ; diagnostic et traitement.** [Epizootic Lymphangitis in France; Diagnosis and Treatment.]—*Rec. de Méd. Vét.* 1916. Apr. 30. Vol. 91. No. 8. pp. 136–142. With 1 coloured plate.

As a result of the war several diseases, which from their rarity in times of peace had been classified as exotics, have made their appearance in France. Epizootic lymphangitis was observed at the beginning of the campaign in horses belonging to Moroccan cavalry, and there is reason to believe that the disease is African in origin when one considers

its frequency in the French possessions in Northern Africa. The number of cases of the disease that passed through a depôt seems quite inconsiderable, but the diversity in origin of these cases indicates a rather disquieting diffusion. The disease at present also attacks horses which are civilian property and certain stables appear to be severely affected.

The author describes the diagnosis of the disease and its differentiation from ulcerative lymphangitis (due to the Preisz-Nocard bacillus), and sporotrichosis.

*Treatment.*—NÈGRE, TROUETTE, and the author have previously published observations on the treatment of this disease by means of intravenous injections of *arsenobenzol*, which they claimed to have furnished excellent results [*Bull. Soc. Path. Exot.*, 1911; *Ann. Inst. Past.*, 1912]. In current practice its employment, however, presents some difficulty on account of the material necessary for the preparation of the solutions and injections.

*Novarsenobenzol*, which gives exactly the same results as the old arsenobenzol, is on the other hand easy to administer. The author recommends a dose of from 2 to 3 grammes dissolved in 20 cc. of distilled water to be injected into the jugular.

"Care should be taken not to allow the escape of fluid subcutaneously as the substance produces a great deal of irritation and pain in the subcutaneous tissues. The effects of the injection are shown afterwards by the cicatrisation of the original ulcers, the diminution in size of the lymphatics, the appearance of new 'buds' on the diseased lymphatic system, the softening and spontaneous opening of existing 'buds.' The 'bud' seems to react by expelling the parasite.

"In order to appreciate the results of the injection it is as well to wait three weeks. A cure is then assured if the cording of the lymphatics has disappeared or considerably diminished, if they have become painless to the touch, and if the original wound is cicatrised. If, on the other hand, the original wound remains open and the sensitiveness of the lymphatics persists it is better to repeat the injection.

"It is sometimes advisable to puncture, by means of a bistoury or cautery, certain abscesses in which spontaneous bursting would otherwise be too long delayed, and to assist the cicatrisation of extensive ulcers by painting them with tincture of iodine. Considerably granulating ulcers should be powdered over with copper sulphate or liquid chloride of zinc."

The author then gives details of nine cases treated as above, all terminating in complete recovery, except one, where, owing to the advanced state of the disease when first treated, recovery was only partial.

(191) DOUVILLE. **Traitement de la lymphangite épizootique. Essais par le galyl.** [Treatment of Epizootic Lymphangitis. Experiments with Galyl.]—*Rec. de Méd. Vét.* 1916. May 30 to June 30. Vol. 91. Nos. 11–12. pp. 144–151. With 4 plates.

Galyl is a definite chemical compound, an organic arsenio-phosphate discovered by Professor MONNEYRAT (formula $C_{24}$ $H_{22}$ $O_8$ $N_4$ $P_2$ $As_4$). Its employment in human medicine for the treatment of syphilis, relapsing fever, and yaws has been recently studied by various authors, while LAVERAN and ROUDSKY in 1913 published experiments to show that the compound was very effective in infections due to *T. brucei*, *T. evansi*, *T. rhodesiense*, and *T. soudanense*. Galyl has the

form of a yellow powder which keeps indefinitely in a closed flask. Its molecule contains 35 per cent. of arsenic and 7·2 per cent. of phosphorus. It is easily soluble in water, forming a transparent yellow or slightly brownish solution.

The author utilised, to commence with, the system of giving fractional doses; an intravenous injection of 90 cg. or a gramme, in a $\frac{3}{4}$ or 1 per cent. solution, being repeated weekly as a rule. Up to 5 grammes, however, of galyl can be tolerated by a horse of average weight without any risk. At present the author gives a preliminary injection of 2 or 3 grammes of the product, according to the extent of the lesions and the size of the patient, and waits a fortnight before repeating the injection. In cases where improvement occurs the second dose does not exceed 1·5 or 2 grammes; in the contrary event, it is equal to the first injection.

All the cases treated by the author with galyl were chosen from amongst the most severely affected and from those cases where effective cauterisation of the lesions could not be applied without risk. The author then gives a history of 15 cases so treated. Eleven cases were completely cured, the total dosage for each having varied from between 3 to 6 grammes and the duration of the treatment from one to three months. Four animals were killed after a month in hospital on account of the extent and severity of the lesions. In the case of these latter animals, the dose injected did not exceed 3·5 grammes, but the author believes that these animals would have remained refractory to a larger quantity of the product. The animals were slaughtered for economic reasons.

Comparatively, the author has tried treatment with *iodides*, which Teppaz utilised for the same infection in Senegal. A certain amelioration was observed in the case of treated animals, but in no case could a cure be said to have been effected, although a total dosage of 300 grammes had been given to some animals.

Treatment with *novarsenobenzol*, recommended by Bridré, was also thus tried. In 12 cases treated, seven animals appeared to be on the road to recovery after having each received an injection of 3 to 6 grammes in the course of a month. In the case of the other five, the lesions seemed to be stationary in spite of the administration of 5 to 11 grammes of the product.

(192) Velu. **Essai de traitement de la lymphangite épizootique par le novarsénobenzol.** [Treatment of Epizootic Lymphangitis with Novarsenobenzol.]—*Rec. de Méd. Vét.* 1916. May 30 to June 30. Vol. 91. Nos. 11–12. pp. 152–155.

The author has had ample opportunity of comparing the effect of various drugs on the very numerous cases of epizootic lymphangitis which occur in Morocco. Contrary to the indications of Bridré and other workers, who have claimed most favourable results from treatment with novarsenobenzol, the author has found that in his hands this product has given no better results than in the case of the other products hitherto employed, in particular colloidal iodine and biniodide of mercury.

(c) SPOROTRICHOSIS.

(193) BRIDRÉ (J.). **Sur un cas de sporotrichose du cheval.** [A Case of Sporotrichosis in the Horse.]—*Rec. de Méd. Vét.* 1916. Apr. 30. Vol. 91. pp. 113 to 116.

The author describes a case of the above disease in a French army horse imported from North America. Sporotrichosis of the horse was observed for the first time in 1909 in Madagascar by CAROUGEAU, and has been found in America by various authors, but up to the present no cases have been reported as occurring in France.

The following were the lesions presented by Bridré's case :—

"Excoriation of the left eyebrow ; a circular ulcer with edges radially striated at the base of the left ear, and from this ulcer commences a cord-like beaded swelling, with hard and insensitive 'buds,' ending at the entrance to the chest ; several buds opened by means of a scalpel disclosed a thick cream-like pus. The animal was placed in a box when these symptoms were first seen and tested with mallein, subcutaneously, but gave no reaction. On the following days the wounds on the eyebrow and at the base of the ear showed a tendency to cicatrisation."

Microscopic examination of fresh pus showed two oblong bodies only, bearing some resemblance to cryptococci, but not showing the characteristic refractile envelope of these parasites. The material was sown on to Sabouraud's agar immediately and kept at room temperature. After about 12 days numerous colonies of *Sporotrichum* appeared on the surface of the agar.

At first sight the characters of this organism did not appear to differ sensibly from those of *S. beurmanni*. A precise description is promised by the author's friend, PINOY, who has commenced the study of the organism.

The above case was treated by intravenous injections of novarsenbenzol, with successful results.

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## BACTERIAL DISEASES.

### UNDULANT FEVER.

(194) IZAR (Guido). **Mutazioni morfologiche culturali e biologiche** *in vitro* **ed** *in vivo* **del micrococco di Bruce per azioni dei sali di chinino. Nota Preventiva.** [Morphological, Cultural and Biological Changes *in vitro* and *in vivo* of Bruce's Micrococcus, caused through the Action of Quinine Salts. Preliminary Note.]—*Pathologica.* 1916. June 1. Vol. 8. No. 182. pp. 175–176.

By the addition of gradually increasing quantities of quinine salts to media, Bruce's micrococcus can become accustomed to, and grow luxuriantly on, media containing several times the dose that would be bactericidal to the original strain.

Thus acclimatised it displays the following changes :—(A) Morphological—(increase in size, chain and bacillary forms) ; (B) Cultural—(a greater rapidity of growth, a dense whitish film on surface growth) ; and (C) Biological—(loss of agglutinability towards serum immune

against the original strain of micrococcus, increase of non-specific agglutinability, but specific agglutinability towards an auto-immune serum persists).

The sera of individuals who had undergone quinine treatment and were affected with a low form of Malta fever did not agglutinate the ordinary strains of the micrococcus; they, however, agglutinated the micrococcus which had grown for several generations on media containing quinine.

The author believes that his strain acclimatised to quinine resembles the *paramelitensis* strain of NÈGRE and RÉNAUD.

(195) SERGENT (Edm.), NÈGRE (L.) & BORIES (L.). **Epidémie de fièvre ondulante à Arzew et Saint-Leu (Dep. d'Oran, Algérie) en 1915.** [An Outbreak of Undulant Fever in Arzew and Saint-Leu (Dept. of Oran, Algeria) in 1915.]—*Bull. Soc. Path. Exot.* June. 1916. Vol. 9. no. 6. pp. 351–356. With 4 charts.

The department of Oran has always been an active focus of undulant fever although in the departments of Algiers and Constantine the disease has now been reduced to insignificant proportions. In the spring of the year 1915 a further increase in severity of undulant fever, which lent some particularly interesting characters to the circumstances, took place in the localities of Arzew and Saint-Leu. The epidemic broke out from February to May and affected 20 people—13 soldiers out of a total of about 10,000 men, and 7 civilians out of a population of a corresponding number. With the exception of three cases in which the blood was not taken, serodiagnoses were positive—after heating the serum to 56 C. for half an hour—to a high degree: 1/500 and 1/1,000.

The principal symptoms and complications observed in the case of each patient are given by the authors in tabular form.

It is noteworthy that two patients out of 17 had not partaken of unboiled milk or of fresh goat cheese. Six out of 17 did not drink unboiled goat's milk but ate fresh cheese, while 9 out of 17 took raw milk or fresh goat cheese. The duration of the disease varied from 35 days up to 6½ months, and three deaths occurred.

The disease was most serious in the case of the soldiers, since all the deaths took place among them and nearly all the others were incapacitated for a long time.

SERGENT (Edm.) and BORIES found in 1907 that samples of goats' milk gave a positive agglutination in the proportion of 3·3 per cent., and likewise the sera of six other animals (horses, asses, dogs). SÉJOURNANT also made the same observations in 1912. The domesticated animals of this region thus appear to harbour the *M. melitensis* in an enzootic state.

The increase of the epidemic in 1915 was particularly severe, probably because it found a favourable medium in the soldiers who had been rendered more sensitive by change of environment and physical fatigue.

W. H. HORROCKS and the English medical men in Gibraltar have shown that this English possession, contaminated by goats from Malta, contaminated in its turn the neighbouring Spanish towns. In these circumstances, it would appear necessary in the presence of this

persistence of undulant fever in Orania to introduce legislation against the importation into Algeria of goats from Spain, or at least submit them to a careful bacteriological examination.

(b) D'Hérelle's Bacillus for Extermination of Locusts.

(196) Monod & Velu. **Note préliminaire sur la destruction des acridiens par l'emploi des cultures microbiennes. (Laboratoire du Service Zootechnique et des Epizooties au Maroc.)** [A Preliminary Note on the Destruction of Acridians by means of Bacterial Cultures.]—*Rec. de Méd. Vét.* 1916. June 15. Vol. 92. No. 10. pp. 346–348.

D'Hérelle isolated in 1910, from an outbreak of disease among locusts in Mexico, a coccobacillus which was very pathogenic for these insects. The authors have undertaken experiments in certain parts of Morocco with a view to the extermination of locusts by infecting them with this organism, and the results are briefly summarised as follows.

D'Hérelle's method provokes very contagious outbreaks among insects either by the dissemination of broth cultures or by contamination with the aid of infected crickets. The contagious enteritis provoked by the coccobacillus among the crickets behaves in the same manner as other epizootic diseases. After a period of incubation one observes a period in which a variable mortality occurs; then a period of decline sets in with a diminution of morbidity, caused either by attenuation of the virulence of the organism or by increase of resistance of individuals. In the most favourable cases one notices a mortality of 70 to 80 per cent. In other cases the mortality does not exceed 20 to 25 per cent.

D'Herelle's method is very difficult of application, and good results can only be obtained by competent technicians. Moreover, unfavourable atmospheric conditions such as rain, heavy dews, or bright sunlight considerably hamper the efficacity of the method. It would also be difficult to spread pure broth cultures over a large extent of territory.

The results, however, have been very encouraging and the authors are prepared to adopt the method on a large scale when future invasions by locusts occur.

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## DISEASES DUE TO ULTRA-VISIBLE VIRUSES.

(a) Variola.

(197) (1) Monod & Velu. **La variole des porcelets au Maroc. Premiers essais de vaccination.** [Variola of Young Pigs in Morocco. First Attempts at Vaccination.]—*Rec. de Méd. Vét.* 1915. Sept. 15. Vol. 91. No. 17. pp. 570–573.

(2) Velu. **Contribution à l'étude de l'étiologie de la variole des porcelets.** [The Etiology of the Variola of Young Pigs.]—*Ibid.* 1916. Jan. 15 to Feb. 15. Vol. 92. Nos. 1 & 2. pp. 24–27.

(3) Velu. **Recherches expérimentales sur la variole des porcelets.** [Experiments on the Variola of Young Pigs.]—*Ibid*, pp. 28–30.

In these three short essays a description is given of the authors' observations and work on the variola of young pigs in Morocco.

The disease is very common in that country and causes considerable ravages among young pigs.

According to POENARU, the disease has been demonstrated to be due to a filterable virus. BOLLINGER states that it may emanate from either cow-pox or sheep-pox, thus producing local or general symptoms respectively. PEIPER states that small-pox of man is transmissible to the pig, and vice versa. The authors, however, state that no sheep-pox or human small-pox existed in districts where the variola of pigs occurred, and that though no precautions were taken in handling the sick the disease was not transmitted to human beings; and also, laboratory experiments showed that the infection of young pigs with pig variola did not give subsequent protection against the infection with cow-pox virus.

The virulence of the disease is very variable. The mortality ranges between 5 and 80 per cent., and the virulence in the course of several years apparently undergoes increasing modifications, that is exaltations and attenuations, giving rise to various degrees of illness (benign, generalised, and septicaemic forms). The reason for this is not known. The disease occurs in all seasons, but more especially in winter. The virus is known to be preserved in the dried-up crusts that are shed from the skin, and this probably explains the annual re-appearance of the variola in infected piggeries during the farrowing season.

Up to the age of 3 or 4 weeks, young pigs are so susceptible that nearly all are attacked during an outbreak. After the age of 2 months they often remain insusceptible, whilst one very rarely meets infections of adults. In the latter case, the disease is always very benign and localised about the teats and ears, and is caused, no doubt, by the sucking pigs.

The rabbit, the guinea-pig, and the rat are immune.

Most of the body tissues of an infected animal are virulent, but the cutaneous lesions seem most apt to transmit the infection. The virus is abundant in the eruptions immediately papules are formed, and it still exists when the secretions become purulent.

The patient itself is the most important factor in the transmission of the disease, but this does not explain the appearance of outbreaks with no direct history of original contagion or apparent importation. It sometimes appears that dust may be an important factor, and the authors have known instances where outbreaks were spread through the country in the direction of the prevailing wind.

The period of incubation is not known. Following variolisation the first lesions appear in three or four days. In natural cases, however, pigs are doubtlessly infected by ingestion or inhalation, and thus the incubative period would be a good deal longer.

*Symptoms.*—Young pigs as a rule present grave general symptoms of depression, wasting, high fever, and sometimes diarrhoea. The vesico-pustular eruptions, which are more or less generalised on the skin, are evolved in 15 to 30 days and are rather haemorrhagic in character. The evolution of the skin lesions corresponds with that of human small-pox. Often, vesicles appear about the eyes and conjunctival mucous membrane, and may involve loss of the eyes. The lymphatic glands of the head and neck are very enlarged and softened. When the disease persists in a place, one often notices subcutaneous pustules about the size of hazel nuts containing creamy pus, and in

the region of the neck these sometimes may become as large as hens' eggs and involve the periosteum of the adjacent bones, later forming persistent fistulae.

The disease ordinarily lasts 1½ to 2 months in each centre of infection. There also exist septicaemic forms in which the disease runs a very rapid hyperacute course lasting only two or three days, and in which one only finds localisations about the head and mucous membranes, especially of the eyes. In fifteen to twenty days the whole of the young pigs in a place may be destroyed.

On post-mortem examination, one finds, in nearly all cases of the disease, a lobar pneumonia with abundant oedema.

When the disease breaks out it is impossible to predict the probable duration of the outbreak. After each case, the animals which have shown symptoms or have developed unnoticed pustules remain a source of infection. When the disease is localised it is always cured. When the eruptions become confluent and involve the mucous membrane the prognosis is very serious. It is always fatal in septicaemic forms. A certain number of infected animals show secondary symptoms, waste away, and die from lung complications. All animals which are seriously affected and recover remain for a long time "bad doers."

*Preventive Inoculation or "Variolisation."*—The authors find that the best material is obtained from vesicles three days after the commencement of symptoms. Before the second day no result is obtained, and later the products are purulent. The lymph is taken from a not too severe case of the disease. Conditions in the field are unfavourable for the preservation of the virus, hence the lymph pulp from the vesicles is inoculated immediately.

The seats chosen for inoculation are the inside of the thighs or the region of the folds of the groin. These are lightly scarified and the pulp applied. Three days after scarification the typical pustules of a benign variola appear and these always remain discrete and strictly localised. After recovery young pigs become immune.

(198) Harde (Edna S.). **Some Observations on the Virus of Vaccinia.** —*Ann. Inst. Pasteur.* 1916. July. Vol. 30. No. 7. pp. 299–306.

"*Summary.*—A pure active virus of vaccinia can be obtained by repeated partial disinfection with carbolic acid and glycerin.

"The virus of vaccinia incubated in tissue cultures composed of plasma and cornea or testis from normal rabbits or guinea-pigs shows a definite increase, but the degree of multiplication is not comparable to that observed in cultures of rapidly growing bacteria. The increase of the virus occurs mainly in the tissue, but very little in the surrounding plasma.

"The multiplication of the virus occurs without a corresponding development of vaccine bodies in the preparations.

"There is no growth of the virus in preparations containing cornea killed by freezing or by hypotonic salt solution.

"There is no evidence of the growth of the virus in preparations in which pieces of paraffin, heart, liver or kidney have been substituted for the cornea or testis.

"The virus is soon rendered inactive in preparations containing plasma and cornea obtained from an immune rabbit. The greatest lytic action is exerted by the plasma.

"Attempts at confirming Fornet's cultural experiments have been unsuccessful.

"Cultural experiments with other media and methods have also given negative results, although the virus has not been killed by incubation for eight weeks at 33° C."

(b) Rabies.

(199) Carpano (Matteo). **Su di un metodo rapido di colorazione dei corpi di Negri nella rabbia e sulla speciale struttura che si mette in evidenza col metodo stesso.** [A Rapid Method of Staining Negri Bodies and the Special Structure of these Bodies brought out by this Method.]—*Clinica Vet.* **1916. June 15–30. Vol. 39. Nos. 11–12. pp. 347–359. With 2 plates comprising 38 figs.**

After reviewing the methods hitherto employed for staining Negri bodies, Carpano details the following technique.

Smears from the hippocampus are fixed either in absolute alcohol or, better, in Zenker solution. In the latter they are left for from four to five minutes, washed and again placed for a few minutes in iodised alcohol.

For the preparation of sections small pieces of hippocampus about ½ cm. in thickness are taken. To obtain the quickest results, they are fixed by placing them in acetone, which should be changed two or three times in the course of about two hours and kept at 37° C. The pieces are then clarified in xylol for 10 to 15 minutes and then placed in paraffin, fusible at 50° to 52° C., and kept at 55° C. for about an hour. Absolute alcohol can be used instead of acetone but fixation takes about double the time.

In order to study the histology of Negri bodies the best results are obtained by fixing in Zenker's fluid for four or five hours, then carefully washing in running water, and afterwards immersing for an hour in diluted iodised alcohol; the piece is then put in xylol at 37° C. for an hour, and then for two hours in paraffin wax fusible at 50° to 52° C, heated to 55° C.

Staining.—The following solutions are employed:—

(1).—*Eosin.*

| | |
|---|---|
| Eosin red (ethyl) or, better, eosin yellow .. | 1 gramme. |
| Distilled Water .. .. .. .. | 100 cc. |

(2).—*Crystal Violet.*

| | |
|---|---|
| Crystal violet .. .. .. .. .. | 1 gramme. |
| Alcohol (95 per cent).. .. .. .. | 20 cc. |
| Dissolve and add a solution of 2 per cent. phenol in distilled water .. .. | 500 cc. |

(3).—*Iodine.*

| | |
|---|---|
| Iodine .. .. .. .. .. .. | 1 gramme. |
| Potassium iodide .. .. .. .. | 2 grammes. |
| Distilled water .. .. .. .. | 400 cc. |

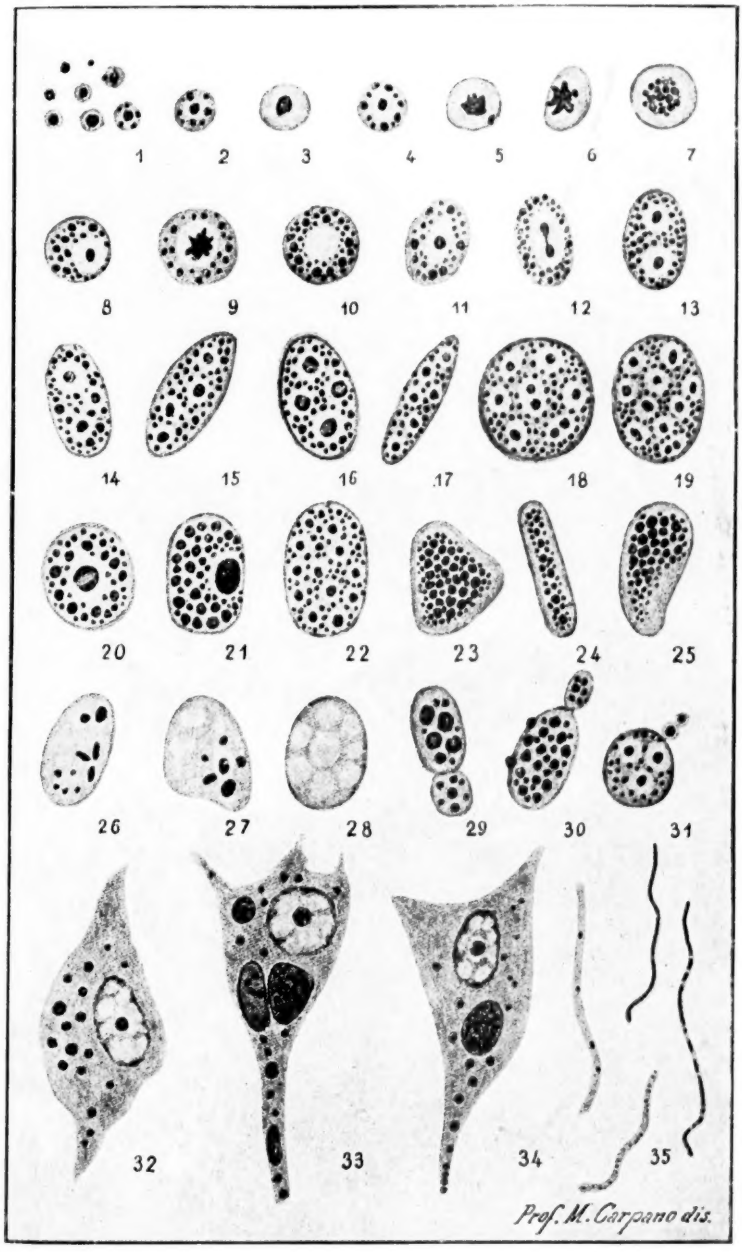
The following are the directions for staining:—

(1) About 10 drops of eosin (Solution 1) are placed on the smear or section for about one minute, run away, and without washing passed quickly into 95 per cent. alcohol.

(2) Before the slide is allowed to dry it is covered with about 10 drops of the crystal violet (Solution 2); the preparation is gently warmed until vapours begin to rise for about five minutes.

(3) The above stain is shaken away from the slide and without previous washing a few drops of the iodine (Solution 3) are put on and left for one minute.

(4) The last named fluid is then also shaken away and the preparation placed for a few seconds in 95 per cent. alcohol until the violet colour has almost completely disappeared.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(5) The preparation is passed quickly through absolute alcohol, clarified in xylol and mounted in Canada balsam.

By this method of staining the cellular elements appear more or less pale pink. The Negri bodies stand out distinctly as they stain of a very dark, decisive, violet colour. Under the low power of the microscope they look almost black, but under moderate and high magnifications they show distinct outlines and a granular interior which is not very clear on account of the intensity of staining.

Better internal differentiation, which is not necessary when staining for diagnosis, is obtained by substituting for the eosin solution a 1 per cent. solution of methylene blue. The specific bodies are then stained dark violet on a cellular basis which is pale blue.

The only elements which are stained in the same way as the Negri bodies are the nucleoli of the great pyramidal cells; these, however, are very easily distinguished on account of their not very distinct outline, constant size, and position in the interior, and often in the centre, of the cell nuclei.

The advantages claimed for this method of staining, besides its rapidity are (1) that it brings out the smallest forms of Negri bodies which are invisible by other methods, and (2) that it reveals their particular structure. One can see a progressive growth and follow a very probable evolution of the structures, and this tends to show that the bodies themselves are of a parasitic nature.

This growth and evolution can best be seen from the following reproduction of one of the Plates.

### Explanation of Plate.*

1. Negri bodies in sections and smears of hippocampus of rabid dogs. Stained by the above method, drawn exactly as seen under the microscope and arranged according to probable development. Figs. 1 to 31 are magnified about 2,000 diameters; the remainder are magnified 800 times diameters.

Fig. 1. Initial forms and forms in the act of developing.

Figs. 2 and 4. Bodies in which granules derived from the primary nucleus are already abundant.

Figs. 3, 5 and 6. Elements in which the primary nucleus has remained undivided and acquired a different appearance.

Figs. 8 and 11. Forms in which the primary nucleus has become surrounded by an appreciable colourless halo.

Fig. 9. Element with central nucleus in form of star.

Figs. 7 and 10. Incomplete figs. as sections were not cut through centre of bodies.

Figs. 12 and 13. Forms in which the central nucleus appears in the act of dividing or else completely divided.

Figs. 14 to 22. Bodies in which rapid division of the nucleus has taken place; the greater part of the small nuclear masses are surrounded by clear halos.

Figs. 23 to 25. Elements in various forms containing large quantity of granules of various sizes.

Figs. 26 and 27. Bodies with a few nuclear granules irregularly distributed.

Fig. 28. Forms showing absence of nuclear masses and a distinctly vacuolated appearance.

Figs. 29 to 31. Bodies apparently undergoing gemmation. Fig. 30 shows at the same time the probable extrusion of nuclear granules.

Figs. 32 to 34. Large pyramidal cells containing Negri bodies in various forms and sizes; in the dendrites will be noticed more or less well-developed elements some of which are very small and arranged in beaded form.

Fig. 35. Filaments observed between the nerve cells, with contents showing various appearances.

* Reproduced by permission of the Editor of *La Clinica Veterinaria.*

(c) Equine Infectious Anaemia.

(200) Theiler (A.) & Kehoe (D.). **Infectious or Pernicious Anaemia of Equines in South Africa.**—*Union of South Africa. Dept. of Agric. 3rd and 4th Reports of the Director of Veterinary Research.* 1915. Nov. Pretoria: Govt. Printing & Stationery Office, pp. 215–289.

Prior to the experiments described in this article by the authors, the above disease was not known to exist in South Africa. It was probably confounded with " biliary fever " (Nuttalliosis, or equine piroplasmosis), or horse sickness, especially in the form observed when an animal had passed through a previous attack and gained a relative amount of immunity, and probably also with the " ephemeral fever " described by Theiler.

The manner whereby the authors came to recognise the existence of infectious anaemia is described by them in the protocols of a number of exceedingly involved experiments. These experiments were originally carried out with a view to ascertaining the relative virulency of some newly collected strains of horse sickness, in order to find out which would be the most suitable in the immunisation of horses against horse sickness, and also to see which strain was the most virulent. This was done by intravenous inoculation into horses which had passed through one or more attacks of horse sickness (immune animals), and horses which had repeatedly received very large doses (10 litres of horse sickness blood intravenously) (hyper-immune horses).

Seventy-six hyper-immune, and four immune horses were available for these tests.

*Expt.* 1 (*10th Oct.*, 1913.)—The above horses were divided into 10 lots and 10 newly collected strains of horse sickness were tested, by intravenous inoculation of 10 cc. of blood per horse, on respective lots. The results showed that five of these viruses did not cause reactions, while five viruses did ; of these latter, three not only caused temperature reactions but even produced death from horse sickness in one case at least each, and one temperature reaction with " dikkop " each.

*Expt.* 2 (*27th Oct.*, 1913.) (A) The viruses from the reacting horses of Expt. 1 were tested on horses of other reacting groups or lots, and where only a temperature reaction had been shown the virus was tested on a control animal in order to prove its capability of producing horse sickness.

Eight viruses (2nd generation) were thus tested.

It became noteworthy that four out of seven horses which had been inoculated in this manner with a certain virus (McCall 59) in Expt. 1 revealed, in the course of this second experiment, reactions which must be considered due to the first injection in Expt. 1. Temperature reactions which continued for 12 days or more were observed either before, or a day or two after, the second inoculation, (The mother virus, however, when tested on a susceptible animal produced death from horse sickness.) The daughter virus of this strain used in inoculating a group in this second experiment failed even to kill a control animal; it produced temperature reactions in the other animals and death in the case of one, which commenced reacting on the 17th day and died on the 31st, death being at the time attributed to broncho-pneumonia. The tardy reactions produced by this virus

(McCall 59) (used in Expt. 1) in the course of the second experiment the puzzled the authors on account of the clinical symptoms shown by horses, which did not resemble those of horse sickness or biliary fever.

(B) Eight additional new strains of virus were tested on the horses of those groups in Expt. 1 which had shown no reactions. It was found that three of these new viruses were capable of producing death from horse sickness in one animal in each of their respective groups, and in one animal of each group, of producing reactions accompanied by " dik-kop." The others either did not react or else produced reactions the nature of which was not understood at the time by the authors.

*Expt.* 3 (13*th Nov.*, 1913).—The properties of three viruses from the first inoculation and three from the second inoculation, all obtained from horses which had died of horse sickness, and three viruses produced from the second inoculations which had given reactions of varying intensity, were tested in the same way on hyper-immune horses of other groups, using also control animals for the last three viruses.

*Expt.* 4 (21*st Nov.*, 1913).—This was a test of the properties of the McCall virus produced from the second experiment.

The results of Expt. 3 and Expt. 4 showed that certain viruses other than the virus McCall were capable of producing a similar type of reaction and that in these new cases the incubation period might also be a long one (otherwise it would have to be considered too short). For example, the reaction which appeared on the first day after inoculation with a virus (Dunning) in the third experiment should be really considered due to the effects of virus (Onderstepoort) inoculated in Expt. 2, eighteen days previously; reaction which appeared on second day after inoculation in this experiment with virus (York) should be considered due to the virus (McCall 59, 2nd generation) inoculated in Expt. 2 nineteen days previously. Other similar reactions were produced which were unfamiliar to the authors and they concluded that some other disease was the factor in producing these results. Temperature reactions even appeared on the 37th day in controls from Expt. 2, and these animals did not die of horse sickness. When tested, however, later with known horse sickness strains they died of that disease.

Controls were inoculated with blood from eight of the horses that had shown reactions in Expts. 1, 2, and 3, and that had not yet been tested to see whether they were really capable of producing horse sickness. Six died of horse sickness, and of the remaining two, one died accidentally on the 18th day, having shown no reaction prior to death, and the other horse showed a temperature reaction from the 5th to the 8th days but did not die of horse sickness.

*Expt.* 5.—Here a study was made of the properties of three viruses, viz. :—

Dunning, 2nd generation, Martin, 2nd generation, Onderstepoort, 1st generation, by injecting them into horses of Expts. 1, 2 and 3 that had not previously received injections of the same viruses (18, 18 and 20 hyper-immune horses for each virus on 5 : 11 : 13, 6 : 11 : 13, and 17 : 11 : 13 respectively).

In considering the results of this experiment the authors state definitely that they were now dealing with pernicious anaemia. Nearly

all the horses inoculated with the first of these viruses succumbed to pernicious anaemia, some of those inoculated with the second virus died of the same disease, while most of those inoculated with the third virus also gave typical reactions. The date of death varied very considerably, the period intervening between the time of last injection varying from 26 to about 150 days, while a good many apparently recovered.

*Further experiments* were made in order to attempt to transmit the disease to animals of other species than the horse, to test the filterability of the virus, and to transmit the disease by administration of blood of affected animals by the mouth, and to transmit the disease by means of the urine of affected animals. The results of these experiments are given in the following remarks of the authors:—

"The general conclusions that therefore appear to be warranted from the results of the foregoing experiments are, that this disease appearing in horses is capable of being transmitted to other horses by the inoculation of the blood or serum of affected animals; that the virus present in the blood or serum of affected animals is capable under suitable conditions of passing through the pores of a Berkefeld filter; that the disease can be transmitted to the mule and the donkey; that we were unable to transmit it to cattle, sheep, goats, or dogs under the conditions of our experiments; and that it could be experimentally transmitted to horses by the administration through the mouth of blood or urine of affected animals when relatively large quantities of these fluids were used.

"The period of incubation of this disease following inoculation may be as short as five days, but, varying between this and twenty-five days, incubation periods of from ten to fifteen days are not uncommon, and in one instance the length of this period appeared to be thirty days. Further, the length of this period does not seem to be in any direct relationship to the type of disease which follows.

"A point of marked interest, however, to which we wish to draw attention is the relationship existing between this disease and horse-sickness, since it has been suggested by certain European workers that infectious or pernicious anaemia possibly represents a modified or mild form of horse-sickness, a suggestion which appears to have been based on an observation that a horse recovering from pernicious anaemia later proved refractory to infection by a particular strain of horse-sickness virus with which it was injected. The evidence on the point, however, was regarded as inconclusive, and, to our knowledge, no definite evidence for or against this view has since then been brought forward.

"Our experiments bear directly on this point, and furnish evidence to show that this view above referred to is incorrect, since the majority of horses in which the disease was produced in these experiments were horses not only immune to different virulent strains of horse-sickness virus, but animals which had even been hyperimmunized, to the extent of 10 litres given intrajugularly, with the virulent blood of horses, passing through horse-sickness reactions induced by the injection of markedly virulent strains of horse-sickness virus.

"We, therefore, from our experiments cannot support the view that infectious or pernicious anaemia represents a modified mild form of horse-sickness, but are forced to regard the two diseases as entities separate and distinct in themselves.

"Another point which the consideration of our experimental observations bring us to remark upon, and which may be noted here, is the apparent unsuitability of the terms infectious or pernicious "anaemia" as descriptive appellations of the disease, for although the disease is definitely infectious, and anaemia is apparent in the chronic form, still anaemia is not necessarily a well-marked clinical symptom in animals suffering from the more acute form of the disease or in those animals which, though clinically recovered, may yet remain virus "carriers" or "reservoirs."

"This latter observation in regard to the absence of anaemia in acute cases is one to which reference has also been made by other investigators,

but we have here retained the names of infectious or pernicious anaemia to denote the disease as being names by which it is already widely known, and under which it is most commonly referred to in the literature dealing with the subject."

A discussion of the symptoms presented in infectious anaemia of equines is given, the acute, sub-acute, and chronic forms being separately treated. This description would apparently apply to the experimental cases observed by the authors as no record of naturally infected cases is given.

Instances are described where the blood of animals apparently clinically recovered remained infective for a long period.

Drugs such as formalin, iodine, and arsenical preparations exerted no curative effects.

A careful description of the post-mortem appearances of animals which had died from the disease in various degrees of severity is given. No characteristic lesion is, however, discoverable.

The authors devote a considerable amount of work to a study of the appearance of the bone marrow, as European authors and the Japanese Commission had insisted that considerable changes took place in this tissue. The bone marrow from the femur and humerus of 201 animals which had suffered from a variety of diseases were carefully examined. The results showed " that the appearances met with in the bone marrow of animals dying from pernicious anaemia may also be met with in animals coming to post-mortem as a result of various other conditions, and therefore cannot be regarded as especially characteristic lesions of the former disease."

Twelve pages are next devoted to the literature dealing with the disease.

*Problem introduced by the disease into the production of horse sickness anti-serum, and methods used for diagnosis of the disease.*

In naturally infected cases, the other two diseases which commonly affect equines in South Africa, viz., horse sickness, and biliary fever, can be distinguished from infectious anaemia without any great difficulty. Under experimental conditions following the injection of horses, already immune, with horse sickness virus one meets with cases where febrile reactions are encountered which at first present a little difficulty in diagnosis. The further progress of the cases and the presence or absence of repeated febrile attacks and other symptoms of sub-acute or chronic forms of infectious anaemia serve to distinguish the diseases. Ephemeral fever is similarly distinguished.

In the manufacture of horse sickness anti-serum the following precautions are now observed. The temperature of the " virus " horse is taken regularly morning and evening for from four to six weeks or longer before its blood is employed for injecting a " serum " horse. The " serum " horse is also kept under temperature observations the whole time it is used in serum production and even for six or seven weeks following the last hyper-immunisation. The serum obtained from individual " serum " horses is kept in separate vessels for several months before mixing. Should a " serum " horse show a temperature reaction at any time following hyper-immunisation, the blood or serum previously obtained from it is injected into a susceptible horse, and if the disease then appears in the latter animal the serum obtained from the former is rejected and

destroyed. If a number of " serum " horses show suspicious reactions a collective test is first made by injecting a mixture of the suspected sera into a susceptible horse, and if the result of this test should prove to be negative no further test is performed ; if, however, it proves to be positive then further tests are carried out in order to isolate the individual infective sera or serum.

The cost in serum production is thus considerably increased, for whereas previously 67 per cent. of hyper-immunised horses became available for bleeding, now the percentage is further reduced owing to the rejection of animals infected with infectious anaemia.

(201) Carré & Vallée. **Sur l'étiologie de l'anémie infectieuse du Cheval.**—[The Etiology of Equine Infectious Anaemia.]—*Rec. de Méd. Vét.* 1916. Apr. 15. Vol. 92. No. 7. pp. 193–199.

In this note the results of work undertaken in the course of the last 12 years by the authors are summarised, and reference is made to the work of distinguished observers who have since confirmed the authors' original findings incriminating an ultra-visible virus as the causal organism of the disease.

*Seyderhelm (K. R.) and Seyderhelm (R.), and also Ries have recently, however, ascribed the cause of infectious anaemia to a poison (*oestrine*) contained in the bots of the horse (*Gastrophilus equi* and principally *Gastrophilus haemorroidalis*). They arrived at this conclusion because, when aqueous extracts of these larvae are injected into the horse, there are produced either acute fatal accidents or else a progressive chronic disease accompanied by anaemia and fever.

Carré and Vallée were unable to demonstrate this property in bots collected by them, and the Japanese Commission, which undertook the study of the disease, was no more fortunate. They further refuse to admit the unicity of pernicious anaemia of the horse, and that the disease is only verminous in origin.

The following are the main points which they bring forward to prove that there exists in the equine species an infectious anaemia of an inoculable bacterial nature due to a filterable virus.

(1) Nearly all the horses affected with anaemia kept by the authors for their experiments did not contain bots or verminous aneurisms. Whilst the geographical distribution of infectious anaemia is limited, bots and strongyles can be found in abundance in innumerable healthy horses and in places where infectious anaemia has never been known to exist.

(2) The " poison " of bots discovered by K. R. and R. Seyderhelm is resistant to heating in the autoclave for one and a half to three hours and is unaffected by alcohol, acetone, ether, chloroform and iodine trichloride. The virus of infectious anaemia is destroyed by heating to 60° C., and this has been confirmed by Ostertag, Marek, the Japanese Commission, etc.

(3) In their experiments the authors were able to realise five successive " passages " of the infection from horse to horse. It has

* [K. R. & R. Seyderhelm. Die Ursache der perniziösen Anämie der Pferde (*Archiv. für exper. Pathologie u. Pharmakologie.* 1914. Vol. 76. p. 149–201).]

never been demonstrated to their knowledge that an intoxication of verminous origin can thus be transmitted in series. K. R. and R. SEYDERHELM, however, maintain that "oestrine releases without doubt an ultra-visible organism which remains inactive in the horse's body until this poison gives it power of propagating, and virulence." They are thus brought round in a tortuous manner to admit the intimate virulent nature of infectious anaemia. (The role of insects in the transmission of the disease has not yet been worked out.)

### (d) SWINE FEVER.

(202) KING (W. E.) & DRAKE (R. H.). **The Antigenic Value of Spirochaeta Hyos in Complement-Fixation Tests on Hog-Cholera Sera. Studies on Hog-Cholera.**—*Jl. Infect. Dis.* 1916. July. Vol. 19. No. 1. pp. 46–62. With 5 charts.

The antigen employed in these tests consisted of an alcoholic extract of *Spirochaeta Hyos*, obtained from pure cultures of the organism. The latter is stated to be present in the intestinal ulcers, caecal crypts, and external local lesions of animals suffering from hog-cholera and has been previously described by the authors in a number of publications.

The authors insist on a careful titration of the materials used for the test. With this antigen, the authors have performed a series of 115 complement fixation tests. Of these, 22 were with normal hog sera from ten different animals, one was with serum from an animal which exhibited a reaction only following inoculation with virus, six with sera from two convalescent or naturally immune swine, 84 with sera from 24 animals suffering from hog-cholera, and one test each was made with two different lots of hyper-immune serum. The animals suffering from hog-cholera had been infected with virus from five different strains obtained from different parts of the United States.

The results, which are recorded in tabular form, show that haemolysis (−) occurred in all cases in which normal hog sera were used whilst complement fixation (+) resulted in all tests with sera from cholera hogs, except in two cases.

Further experiments showed that complement fixation commenced to appear at about the same time as the clinical symptoms, and that the time of its appearance depended on the virulence of the infective material and the individual resistance of the animal.

Another experiment indicated that the complement-binding substances ceased to exist in the blood when immunity against hog cholera became fully established.

The authors undertook control tests with the following antigens :—(1) *B. cholera-suis* (received from Theobald SMITH), (2) *B. voldagsen* (received from HAENDEL), (3) *B. typhi-suis* (GLAESSER). (4) *Sp. hyos* antigen. Entirely negative results were observed when the first three antigens were used whereas pure *Sp. hyos* antigen gave positive results.

In order to test the specificity of the *Sp. hyos* antigen the authors procured sera from pigs which they had infected experimentally with the following diseases :—(1) *Staphylococcus* septicaemia, (2) *B. cholera-suis* infection, (3) Anthrax, (4) *Ghon-Sachs* bacillus infection, (5) Brine or salt poisoning and pneumonia. The antigen possessed no complement binding properties when brought into contact with the above sera.

(203) Dorset (M.) & Henley (R. R.). **Production of Clear and Sterilised Anti-Hog-Cholera Serum. (Preliminary paper.)**—*Jl. of Agric. Res.* 1916. May 29. Vol. 6. No. 9. pp. 333–338. With 2 tables.

In the United States the anti-hog-cholera serum of commerce for the most part consists of defibrinated blood of hyper-immunised pigs, this practice being adopted owing to the difficulty experienced in separating the serum from the fibrin and the blood corpuscles.

When allowed to undergo spontaneous coagulation one finds in practice that not more than 30 to 35 per cent. of serum can be secured from the blood, the remainder of the serum being held firmly within the large clot.

Owing to the occurrence of foot-and-mouth disease in the United States and the accidental infection of certain lots of anti-hog-cholera serum and virus with this disease, it was found necessary to adopt some method of treating these products which would serve to remove the possibility of either of them being a medium for its dissemination. There would seem to be only one means by which the serum might be sterilised in so far as the virus of foot-and-mouth disease is concerned, and that is by the application of heat. Heating to a temperature of 50° C. for 12 hours kills this virus, and it has been shown that defibrinated hog-cholera-immune blood may be so heated without destroying the anti-bodies or altering the physical character of the defibrinated blood. In practice this process is difficult and expensive to carry out.

When defibrinated hogs' blood is subjected to centrifugalisation (force equivalent to 1,700 times gravity for 20 to 30 minutes), a yield of about 50 per cent. of serum is ordinarily secured. The serum thus obtained is usually cloudy, and owing to the fact that the red blood corpuscles are not firmly packed together it is impossible to remove all the serum without at the same time carrying over some of the red cells.

In order to overcome the above difficulties the authors have used an extract of the seed of different varieties of the common garden bean (*Phaseolus multiflorus* and *P. vulgaris*). Extracts of these beans are known to possess the property of agglutinating the red blood corpuscles of the pig's blood and they are said to be non-toxic. Very minute amounts of the extract of these beans serve to agglutinate large quantities of the defibrinated blood, and when such agglutinated blood is centrifugalised the red cells are packed together forming a rather stiff jelly-like mass and a yield of 50 per cent. of serum may be separated. The serum thus obtained is clear and may be readily poured from the tube.

In order to secure a greater yield of serum and a more firmly packed clot of red corpuscles, the authors further recommend the addition of 1 per cent. of sodium chloride to the defibrinated hog's blood after agglutination caused by the addition of bean extract has begun. This will increase the yield of serum from 50 per cent. without salt to 70 per cent. when salt is added.

The authors recommend the following technique for the preparation of clear sterile serum heated to avoid the possibility of foot-and-mouth disease infection.

*Preparation of bean extract.*—100 grammes of coarsely ground white navy beans are allowed to soak for one hour in 500 cc. of distilled water with occasional stirring; the mixture is then strained, and filtered through a bacteria-proof filter.

*Preparation of defibrinated blood for centrifugalising.*—1 cc. of sterile bean extract is added to each 100 cc. of the cool defibrinated blood, stirred, and then allowed to stand until agglutination commences, which is usually apparent within five minutes of adding the extract. One gramme of finely powdered sodium chloride is then added, stirred in until dissolved, and the mixture is then allowed to stand for about 15 minutes.

*Centrifugalising.*—The defibrinated blood mixture is centrifuged for 15 minutes at a speed sufficient to produce in the cups a precipitating force equal to approximately 1,700 times gravity. The serum is then poured from the cups into suitable containers.

*Heating the serum.*—The vessel containing the clear serum is placed in a suitable water bath maintained at a constant temperature of 60° C. for 30 minutes. The serum is then rapidly cooled and one part of a 5 per cent. solution of phenol is added to every nine parts of serum.

*Filtering the serum.*—After the addition of phenol a slight precipitate sometimes forms in the serum, and it is thus desirable to allow several days to elapse between the addition of phenol and the final filtration through a bacteria-proof filter of infusorial earth.

An experiment in which ten pigs were used demonstrated that the amount of anti-bodies left behind with the red blood corpuscles was negligible, as the latter, when injected subcutaneously even in large quantities, failed to produce any immunity against the hog-cholera virus. Also, there was hardly any difference between the degree of immunity conveyed by the serum alone, and the defibrinated blood. This showed that the serum may be heated for half an hour at 60° C. so as to safeguard against infection with foot-and-mouth disease without any noticeable impairment of its potency.

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## DIETETIC DISEASES, Etc.

(204) Graham (R.) & Himmelberger (L. R.). **Studies in Forage Poisoning. III.**—*Jl. Comp. Path. & Therap.* 1916. June. Vol. 29. Pt. 2. pp. 107–116.

This article is a continuation of the authors' studies on forage poisoning.

Considerable losses were caused among cattle and horses in Kentucky fed during the winter months on silage. Bacteriological examination of this (N.) silage revealed the presence of a micro-organism similar to that previously isolated by the authors from an oat hay, which was shown to possess pathogenic properties.

This micro-organism occurred in the form of a short bacillus 1 to 2 microns long by 0·4 to 0·5 microns broad, motile, spore-producing, staining with the ordinary anilin dyes, Gram-negative, and aerobic. Grows well on agar.

The authors describe 14 experiments on cattle, horses, sheep, goats, and small laboratory animals. In these experiments feeding and inoculation tests were carried out with silage, watery extract of silage, cultures of the bacillus, and sterile filtrates from cultures. The results of these experiments are included in the following conclusions :—

"A bacillus isolated from N silage possessed pathogenic properties towards some experimental animals. We were unable to demonstrate its fatal character for all of our experimental animals. Calves No. 5 and No. 7 furnish evidence of its pathogenicity. The remaining calves and two mature cows are suggestive of the transitory effects exercised by this organism, while horses seemed most susceptible and guinea-pigs and rabbits were apparently immune. We are not in a position to say that similar results could not be attained with other bacteria isolated from apparently poisonous forage or even with bacteria isolated from wholesome forage.

"Daily administrations of sterile filtrates of this bacillus grown on a synthetic medium, introduced intrajugularly, and bouillon cultures in the form of enemas, produced death in horses, as did also daily drenches of bouillon cultures to calves, with clinical manifestations and gross anatomical changes not unlike those observed in some cases of forage poisoning.

"The morphological and cultural features of this bacillus are in every major detail analogous to those possessed by a pathogenic organism isolated from an oat hay responsible for losses among horses and mules, as demonstrated by feeding experiments. The possibility of this bacillus being closely related to the spore-bearing bacillus from a different source, described in a previous publication, is further suggested in experiments on horses by the manifestation of similar symptoms and *post-mortem* lesions after death. A poisonous substance is evidently produced by the bacillus in question, since sterile filtrates of cultures grown on Uschinsky's medium produced death in horse No. 62, as well as other experimental horses not recorded in this paper. The symptoms following intravenous injections are quite sudden and intense, yet not unlike those observed subsequent to intestinal or rectal absorption in calves and horses. In a control experimental horse no unfavourable results followed the daily intravenous injection of similar quantities of sterile uninoculated Uschinsky's medium or daily enemas of sterile bouillon. It is at least a suggestive contribution to our knowledge of forage poisoning that a bacillus may be associated with silage which on being cultivated on synthetic media is found to produce a substance poisonous to horses. The poisonous properties of this bacillus may be extra-cellular, or endo-cellular as a result of cell disintegration. The toxic properties of this bacillus could not be demonstrated by single injections into guinea-pigs and rabbits, using sterile cultural filtrates grown on Uschinsky's medium that proved to be pathogenic to horses. The method used to demonstrate anaphylactic phenomena in guinea-pigs with other foreign protein (egg white) failed. In this connection some anaphylactogens (bacterial) sensitise in very minute doses, while larger and repeated doses are necessary in other cases.

"Difficulty has been reported in sensitising guinea-pigs to bacterial protein. According to Rosenau and Anderson, Vaughan, Holobut, and Kraus and Doerr, a variation of technique in sensitising guinea-pigs seems more reliable than the usual methods employed in sensitising to animal proteins. The limits seem to vary with the protein and the animal. If true anaphylaxis occurred in horses Nos. 48 and 55, it is in further variation of the usual technique employed to demonstrate this phenomenon, as there existed no intervening or rest period subsequent to feeding or sensitising and the injection of the toxic dose.

"Further evidence that the filtrate immediately after filtration does not contain true protein substances are the negative chemical tests with Millon's fluid, xanthoproteic, and Heller's ring test. The uninoculated sterile Uschinsky's protein-free medium gives a positive reaction for protein by the biuret test, as does the sterile filtrate of this bacillus in Uschinsky's medium, but the latter test is not considered reliable for detecting the presence of true protein. Horses gave evidence of hypersensitiveness when injected with N–1 culture filtrate on synthetic medium

subsequent to being fed with the N silage and watery extract of same to drink, and also subsequent to the feeding of O–1 cultures on bouillon, yet death followed daily injections of the sterile filtrate without preliminary treatment, suggesting the poisonous properties of the filtrate *per se*.

"Following the initial injection, manifest disturbances generally occurred in a few minutes. The sudden manifestations following injection preclude the possibility of a true soluble toxin or bacterial infection, but clearly suggest the presence of a substance which exerts a toxin-like effect. The cumulative or hypersensitive-like condition prevailing in horse No. 55 is evidence of the close relationship existing between the filtrate obtained from the bacillus isolated from the silage and the filtrate from the bacillus isolated from the oat hay, feeds responsible for widely separate outbreaks of this disease."

(205) Stockman (S.). **Cases of Poisoning in Cattle by Feeding on Meal from Soya Bean after Extraction of the Oil.**—*Jl. Comp. Path. & Therap.* 1916. June. Vol. 29. Part 2. pp. 95–107.

This article is of interest on account of the very widespread use at present of soya bean products as feeding stuffs for cattle in all parts of the world, and on account of the often alleged cases of poisoning resulting from their use. The experiments described in this article were undertaken to ascertain the cause of deaths amongst a number of cows in the south of Scotland which had been fed on soya bean meal.

The symptoms, which were sudden in their onset, were as follows: Discharge of blood from the nostrils, congestion of the visible mucous membranes, suspension of rumination, shivering, high fever (105° to 109° F), discolouration of the faeces due to blood; symptoms of abdominal pain; presence of subcutaneous nodules, from the size of an egg to that of a child's head, caused by haemorrhages. The interval elapsing between the first appearance of symptoms and death in the case of the 36 animals observed varied from 1 to 17 days.

On post-mortem examination widespread haemorrhages were found on all the mucous and serous membranes of the body. In the small and large intestines these haemorrhages had caused the rupture of the mucous membrane, and appeared like the haemorrhagic ulcers sometimes seen in cattle plague and East Coast fever.

Fourteen experiments were carried out by the author with samples of the extracted soya bean cake and meal on 8 heifers, 2 pigs, 1 ewe, 3 guinea-pigs, and a rabbit. The author summarises his experiences as follows:—

"1. The cases produced at the laboratory by feeding on extracted soya cake and meal are identical in symptoms and *post-mortem* lesions with the cases occurring in the field amongst cattle which were fed on similar material.

"2. In both cases the animals were receiving other food-stuffs (some went out to grass). This discredits the possibility of the trouble being due to a deficiency in vital constituents, as in the scurvy type of disease.

"3. In both cases the trouble sometimes did not show itself for one or two weeks after the extracted soya meal or cake had been discontinued and the animal had been turned out to grass. This is a curious feature, and points to the poison being one which takes some time to act, although a poisonous dose is present in the system. It might also mean that the actual poison is manufactured inside the animal by a slow process from extracted soya (*see also* 4).

"4. The extracted soya in no case (practice or laboratory) produced a sudden effect; in all a considerable amount was consumed, and a considerable time elapsed before signs of illness appeared. The smallest

amount consumed at the laboratory before disease began was 172 lbs. (thirty-six days); the shortest time in which disease appeared was twenty-nine days (201 lbs.).

"5. No species other than cattle suffered from feeding on this meal or cake, either in practice or at the laboratory.

"6. The very high temperature (106° to 109° F.) accompanying the illness seems to exclude the ordinary poisons, but does not exclude a poison of the ricin class. Specific bacterial infection was excluded by test inoculations, microscopical and bacteriological examinations, and by the fact that a sterilising temperature was used in the process of manufacture. No castor seeds could be traced in the meal.

"7. From inquiry (very wide) whole soya bean is not poisonous.

"8. From inquiry amongst manufacturers there is plenty of evidence that soya extracted with naphtha does not cause poisoning, and it would appear that trouble followed the use only of soya extracted with trichlorethylene.

"9. Trichlorethylene itself, however, is not poisonous when given to cattle in comparatively large doses—1 to 3 ozs., and for long periods. It may be: (*a*) that the products from trichlorethylene obtained by heat are poisonous (this is doubtful); (*b*) that the trichlorethylene in contact with the soya and heat used to drive off the former forms a poison; or (*c*) that some of the trichlorethylene was impure and contained other bodies.

"10. It would appear from what occurred in practice that all bovines are, at least, not badly affected by the poison. On nine premises upon which trouble occurred the percentage of visibly affected varied from 1 to 19 per cent.—average, 10 per cent. The farmers thought, however, that the other animals suffered also, though not visibly. It is not improbable, of course, that this was so (*see also* Experiment III), and that it was only the very badly affected which were taken as being ill. Amongst the visibly affected the death-rate varied from 6 to 100 per cent.—average, 84 per cent. It is possible, having regard to the above, that the poison is one against which a certain amount of immunity can be acquired, as in the case of ricin, but such an immunity did not show itself in Heifer 313.

"11. If it be that, in order to obtain a poisonous quantity of the active agent, some 200 lbs., or more, of the material must be extracted, it would hardly seem to be a process which could be undertaken in an ordinary laboratory, but would require a small manufacturing plant.

"12. If it be that the active agent is elaborated from the extracted soya in the digestive organs of bovines the problem of isolation becomes still more difficult.

"13. If 12 be assumed, the most promising method of search might be to produce cases experimentally, and then try to extract a poison from the organs.

"14. Extracted soya meal constitutes an excellent auxiliary foodstuff for cattle, but it is inadvisable to use trichlorethylene as the extractor."

(206) Theiler (A.), Green (H. H.) & Viljoen (P. R.). **Contribution to the Study of Deficiency Disease, with Special Reference to the Lamziekte Problem in South Africa.**—*Union of South Africa. Dept. of Agric., 3rd & 4th Reports of the Director of Veterinary Research.* 1915. Nov. pp. 9-68. Pretoria: Govt. Printing & Stationery Office.

The scheme of work considered in this communication comprises:—

"(*a*) Rice-feeding experiments upon cattle, horses, sheep, goats, and pigs, with the object of producing, if possible, deficiency disease in the animals concerned, and of comparing the symptomatology of such disease with gal-lamziekte.

"(*b*) Experiments with pigeons with the primary object of testing various rations used in the other experiments, in respect to their anti-neuritic efficiency or vitamine content.

" (*c*) Experiments on dogs with the object of inducing an avitaminosis by rice-feeding, and of testing the effect of feeding flesh of cattle which had died from lamziekte.

" (*d*) Feeding experiments upon cattle running on the lamziekte camps at the experimental farm, Armoedsvlakte, near Vryburg, Bechuanaland.

" In this case the object was to determine whether or not lamziekte, in its natural incidence, could be (1) controlled prophylactically by the use of supplementary rations of known high vitamine content, (2) successfully treated with vitamine extracts, (3) increased by partially substituting polished rice, known to be deficient in vitamines, for the natural pasturage, (4) influenced by factors which affected the condition of the cattle without seriously affecting the vitamine content of their diet or the extent of natural grazing.

" (*e*) Feeding experiments with hay cut at random from lamziekte camps, with the object of eliminating as far as possible all factors other than the purely dietetic.

" (*f*) An attempt to co-relate the incidence of disease with the rainfall and condition of the veld on the area concerned, in respect to hypothetical vitamine deficiency of the natural pasturage."

The results of an enormous amount of work are described which throw a good deal of light on the " vitamine " problem. The authors failed to connect the causal agent of lamziekte with any " vitamine " deficiency in the feeding stuffs. A careful perusal of this article would be of great interest to students of physiology and dietetics.

## Summary and Conclusions.

" The primary economic purpose of the investigations detailed in the text was to obtain evidence for or against the hypothesis that the South African disease known as lamziekte or gal-lamziekte, a neuro-muscular fatal complaint of cattle, is an avitaminosis. Correlated to this was an attempt to produce an avitaminosis in various classes of animals and to obtain data of general interest in scientific dietetics. The evidence incidentally acquired suggesting other theories of causation, is not specifically considered.

" Pigeon-feeding tests were carried out to determine the anti-neuritic properties of various foodstuffs, more particularly those fed as supplementary rations to cattle naturally grazing over lamziekte veld suspected of vitamine-deficiency.

" On the basis of these tests, different batches of cattle on the affected area were supplied with vitamine in the form of beans, bran, maize, potatoes, and yeast, in amount in large excess of the theoretical quantity required for the metabolism of the gross food eaten. At the same time another batch was supplied with a liberal ration of polished rice with the idea of still further reducing the vitamine-content of the natural diet. The mortality in the batches receiving supplementary vitamine-rich rations and in the batch receiving polished rice, was the same as that of the control animals, and the conclusion was therefore drawn that the prevalence of lamziekte bears no relation to the vitamine-content of the natural pasturage.

" Kraaled cattle fed on hay cut at random from lamziekte areas did not contract the disease, and in so far as the limited nature of the experiment allows of a conclusion, the disease would appear to be non-dietetic (*in the nutritional sense*) in origin.

" No clear relation of rainfall to prevalence of the disease on the experimental area could be detected.

" No clear difference in mortality could be detected amongst cattle in good condition and those in poor condition. An average mortality of about 30 per cent. in the experimental cattle prevailed independently of feeding or condition.

" Therapeutic treatment with vitamine extracts, even in theoretically excessive amounts, and prophylactic treatment with yeast, proved useless.

"Cattle fed for periods up to thirteen lunar months on synthetic rations of exceedingly low vitamine-content, as judged by accepted criteria and pigeon analysis, failed to develop either lamziekte or any specific disease which could be diagnosed as an avitaminosis. This is regarded as strong corroborative evidence against the vitamine-hunger theory as applied to lamziekte, and the view is incidentally expressed that an avitaminosis in cattle is not likely to occur in practice unless manifested simply in an atrophic form clinically indistinguishable from inanition.

"Horses, fed upon diets similar to the polished rice rations fed to cattle, also developed no symptoms of specific deficiency disease within periods of six months, and the conclusion is drawn that an avitaminosis in the horse, if it exists at all, is a matter of slow development, and would tend rather to be obscured by preceding inanition, on any naturally occurring diet upon which the animal could conceivably be fed.

"Dogs fed on polished rice succumbed, after periods varying up to four months, under symptoms suggestive of generalized malnutrition rather than those of a specific clinically recognizable avitaminosis of either the beriberi or scurvy type. Specific rather than general deficiency is, however, regarded as the cause of death, although the calorific intake was not sufficiently accurately ascertainable to settle the point.

"Lamziekte biltong showed it was probably as efficient as ordinary biltong in enabling dogs to maintain equilibrium on a polished rice diet, and in restoring dogs, emaciated after rice feeding, to normal health. These experiments are regarded as analogous to pigeon tests in which no marked constant difference in vitamine content could be detected between dried flesh from healthy animals and that from diseased animals. The dogs showed no ill-effects as a result of eating the diseased biltong.

"Pigs, goats, and sheep, fed for prolonged periods on rations in which polished rice was either the exclusive or the preponderating constituent did not develop any specific deficiency disease. No definite conclusion, however, is drawn from these experiments, since the protocols are open to interpretation in more than one way.

"A number of points in connection with the problems of growth and general dietetics are raised."

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## THERAPEUTICS.

(207) Green (H. H.). **The Sulphur Sheep Dips.**—*Union of South Africa. Dept of Agric., 3rd & 4th Reports of the Director of Veterinary Research.* 1915. Nov. pp. 113–162. With 3 plates comprising 13 figs. Pretoria: Govt. Printing & Stationery Office.

In this article the chemical aspect of the sulphide dips are dealt with in the following order :—

(1) Caustic soda and sulphur dip.
(2) Lime and sulphur dip.
(3) Loogas and sulphur dip.
(4) Efficacy for the cure of scab.
(5) Probable decomposition of the sulphide dips in the fleeces of the sheep.
(6) Effect upon the skin and wool of sheep.

The following is the author's summary :—

"1. The caustic soda and sulphur dip, as prepared according to the recommendations of the Division of Sheep, consists of a mixture of sodium pentasulphide and sodium thiosulphate with a distribution of sulphur

about four-fifths of the former and one-fifth of the latter. No monosulphide can be detected and only traces of sulphate are present. There is even the suggestion that polysulphides higher than the pentasulphide exist for a short time in the freshly prepared dip. The proportions of caustic soda and sulphur actually going into combination are approximately five to eight, and the recommended formula, 5 : 20 : 2½–100, provides so large an excess of free sulphur that complete utilization of caustic soda is ensured even under somewhat careless conditions of dip-making. The presence of free caustic soda in the dip need not therefore be feared if the instructions for dip-making are carefully carried out. The preliminary mixing of the sulphur to an homogenous cream with water is the most important step to be observed, since it is upon the intimacy of subsequent contact of the sulphur with the caustic soda solution that the reaction mainly depends. Combination takes place at comparatively low temperature, and may be completed in forty minutes at 50° C., although reaction is of course more rapid at higher temperatures. If hot water is used in the preliminary mixing, the heat evolved as the caustic soda is sprinkled in suffices to keep the mixture near the boiling point, and boiling by the application of external heat is therefore unnecessary. Boiling, however, may offer slight advantages in completing the reaction in cases where the preliminary process has been imperfectly carried out. With ordinary care a dip of correct composition always results, without boiling. If the sulphur is not properly wetted in the preliminary mixing to a cream, the bulk of the sulphur may float on the surface and cake as the caustic soda is sprinkled in, and so partially escape combination. The dip may then contain residual free alkali, but not, as might be expected, any appreciable amount of sodium monosulphide.

"2. The lime-sulphur dip is analogous in composition to that of the caustic soda and sulphur dip, and consists of a mixture of calcium pentasulphide with calcium thiosulphate. In preparing the dip the ingredients must be boiled, and the so-called 'raw lime-sulphur dip' consists merely of a mixture of lime and sulphur. At least two parts of sulphur to one part of unslaked lime should be used whenever a really good sample of lime is available. If other proportions are used, the material present in smaller amount determines the solution of the other, higher polysulphide rather than lower being formed in all cases. If the lime is in excess, small quantities of free hydroxide may be present in the dip, but the amount is limited by the low solubility of the lime itself. Calcium pentasulphide and calcium hydroxide can, however, co-exist in the same solution even at the boiling point.

"3. The loogas-sulphur dip consists mainly of a mixture of carbonates, polysulphides,and thiosulphates, of sodium and potassium, the amount of polysulphide being very low and the amount of carbonate relatively high. The reaction between alkaline carbonates and free sulphur is very imperfect even after prolonged boiling, and most of the sulphur used in making the loogas dip is therefore wasted by passing into the unused sediment.

"4. The kind of sulphur used in dip-making is of no consequence provided it is finely divided and fairly pure. Flowers of sulphur and ground rock sulphur are equally suitable, but in the latter case a guarantee of fineness of grinding should be demanded. 65° Chancel is suggested as a reasonable specification.

"5. The quality of lime used is obviously of paramount importance in making the lime-sulphur dip, but limes generally available in the Union appear to be of very inferior grade. The saving grace in cases where bad limes have been used in practice, lies in the fact that concentrations of poly-sulphide much below that obtained in a well-made dip are still effective in curing scab.

"6. The lime-sulphur and caustic soda and sulphur dips are reliable for the cure of scab. The loogas-sulphur dip also cured scab in the experimental trial carried out, but its composition suggests that its efficacy is largely a matter of chance, and that it is therefore not to be relied upon.

"7. The active constituent of the sulphur dips appears to be the polysulphide, since thiosulphate, the only other important constituent is itself

ineffective. Free base, if accidentally present, does not contribute to the parasiticidal efficacy of the dips. A concentration of 0·6 per cent. sulphur in polysulphide form is probably always high enough to effect a cure. 0·3 per cent. showed itself as uncertain in action. The polysulphide content of the home-made lime-sulphur dip is much higher than that of the caustic soda and sulphur dip, but it is not advisable to dilute the home-made dip further than is already customary unless the lime used in making is known to be of very high quality. If commercial lime-sulphur concentrates are used, dilution may be conveniently carried down to a concentration of about 0·8 per cent. polysulphide sulphur.

'8. The sulphur dips if properly made (almost neutral to phenolphthalein) are harmless both to the sheep and to the wool. Solutions of polysulphide, at the concentration used in dipping, have no action on wool even on prolonged steeping. On the fleeces of dipped sheep polysulphide is rapidly and almost quantitatively converted into thiosulphate by atmospheric oxidation, and this occurs long before the fleeces are dry. No depilatory substances are formed in detectable amount, as intermediate products in normal decomposition. If, however, free base be present in large quantity along with polysulphide it is possible for monosulphide to be formed as intermediate product in the course of atmospheric oxidation, and the possibility of depilatory action then arises. Minor quantities of free hydroxide, up to about 10 per cent. of the total base present in the dip, appear to be of no practical consequence, since they are converted into thiosulphate during atmospheric oxidation. The depilatory action of monosulphide or hydrosulphide is much more violent than that of free hydroxide.

"Under ordinary circumstances a dip would have to be very badly made indeed before the amount of residual base present could become a source of danger in practical dipping, and if reasonable care is taken in preparation the possibility of injurious action either upon the sheep themselves, or upon their wool, is altogether ruled out.

"Although dyeing or spinning tests could not be carried out at this laboratory, it appears highly improbable that the expert dealer in woollen fabrics could tell the difference between a washed fleece previously dipped in the sulphur dips and a washed fleece previously dipped in any of the ordinary proprietary dips."

(208) Green (H. H.). **Upon the Composition and Analysis of Polysulphide Solutions.**—*Union of South Africa. Dept. of Agric., 3rd & 4th Reports of the Director of Veterinary Research.* 1915. Nov. pp. 173–195. Pretoria: Govt. Printing & Stationery Office.

"1. A general discussion of the validity of the iodine titration method of Harris for the analysis of polysulphide mixtures is offered, and preference is given to the older methods involving the use of ammoniacal zinc chloride. In the presence either of free lime or free sulphuretted hydrogen, the iodine titration method countenanced for the analysis of lime-sulphur solutions, by the American Association of Official Agricultural Chemists, is altogether invalid, but by combining the ammoniacal zinc method with simple titration to standard acid, reasonably accurate results may be obtained, whatever be the composition of the polysulphide solution.

"2. The recent views of Ramsay and of Auld in regard to the nature of lime-sulphur solutions and to the reaction involved in their manufacture are discussed and criticized as invalid. Apart from the presence of thiosulphate, the dominant constituent is calcium pentasulphide, and 'polysulphide sulphur' is regarded as firmly combined. Even if huge excess of free lime over sulphur be used in preparation, the tendency always is towards formation of higher rather than lower polysulphides, the higher derivatives being stable even on boiling with free base.

"The possibility of the existence of derivatives above the pentasulphide is also discussed."

(203) Bedford (G. A. H.). **Report upon the Dipping Trials carried out with the Different Proprietary and Home-made Sheep Dips in South Africa.**—*Union of South Africa. Dept. of Agric., 3rd & 4th Reports of the Director of Veterinary Research.* 1915. Nov. pp. 163–172. Pretoria: Govt. Printing & Stationery Office.

Summary and Conclusions.

"Practical trials on a small scale were carried out with the following sheep dips available in South Africa:—

"(1) Home-made Lime-sulphur Dip, (2) Capex Lime-sulphur Concentrate, (3) Modderfontein Lime-sulphur Concentrate, (4) Home-made Caustic Soda and Sulphur Dip, (5) O'Gorman's Liquid Sulphur Dip, (6) Home-made Loogas-sulphur Dip, (7) Little's Fluid Dip, (8) Hayward's Paste Dip, (9) McDougall's Powder Dip, (10) Cooper's Sheep Dipping Powder, (11) Arsenite of Soda and Sulphur Dip, (12) Jeyes's Fluid, (13) Kerol, (14) Leach's Sheep Dip, (15) McDougall's Tobacco Dip, (16) Dreadnought Tobacco Dip, (17) Magic Sheep Dip, (18) Home-made Tobacco Dip.

"From six to twelve sheep, very badly infected with scab, were used in each test. In the main series of trials two dippings at an immersion period of two minutes, and a dipping interval of nine days, were carried out—the nine-day interval having been previously established as just within the life-cycle of *Psoroptes communis*, var. *ovis*, in South Africa.

"The different batches of dipped sheep were tested for eradication of scab by being kept under observation in isolation stalls for six months after dipping.

"The general results showed successful cure of scab in all trials except one. The exception is represented by O'Gorman's dip, but the failure in the case of one sheep is doubtless due to too high a dilution of the drum fluid as recommended in the directions for use. At higher tank strength this dip would also be effective.

"No attempt was made to place the various preparations in order of merit. This would have involved more extensive trials on a larger scale, and in any case it was not desired to draw invidious distinction between different proprietary articles.

"A few tests carried out at shorter immersion period and longer dipping interval indicated that, although such conditions might be effective, they could not be regarded as safe—especially in the absence of auxiliary hand-dressing, where time must be allowed for penetration of scab crust and where the danger of development of original infection is greater.

"The effect of the various dips upon the general health of the sheep, and upon the skin and wool, was not observed to be unfavourable except in the case of Leach's dip, where obvious intoxication occurred in ten out of the twelve sheep dipped. The deaths occasioned in this case are attributed to the high concentration recommended for use. At lower concentration of tank fluid and with revised instructions for use this dip would probably be efficacious and innocuous. In regard to the recent controversy concerning the home-made sulphur dips there is every indication that these, if properly used, are both effective and harmless.

"As final conclusion it may be safely asserted that, with the exceptions indicated, *all the dips tested are permissible and effective in the cure of scab if properly used at an immersion period of two minutes and a dipping interval of nine days. Single dipping or dual dipping at a longer interval than nine days, or immersion of less than two minutes, may be effective in some cases, but cannot be relied upon.*

"The tests recorded are to be regarded as experimental in nature, and as serving as basis for more extensive trial in the field. The most interesting point in the laboratory trials is the apparent ease with which scab can be cured with almost any accredited dip."

(210) VELU. **Le traitement mercuriel chez le cheval et l'alopécie.** [Mercurial Treatment in the Horse and Alopecia.]—*Rec. de Méd. Vét.* 1916. Apr. 30. pp. 134–136.

The author recently treated 40 horses and mules affected with epizootic lymphangitis by means of intramuscular injections of biniodide of mercury; the doses administered ranged from 1 to 3 grammes in all for each animal, or 10 centigrammes were given every alternate day over a period of 60 days.

In no case was observed the slightest sign of hydrargyrism.

One subject only showed the classical symptoms of mercurial alopecia. This horse received the above treatment on alternate days. A month after the commencement of the treatment a considerable amount of oedema was noticed under the sternum, which extended towards the abdomen and the upper parts of the fore limbs. On the following days the usual train of symptoms exhibited by mercurial alopecia was observed, viz., an erythematous exudative dermatitis, loss of hair between the fore legs, on the shoulders, on the sides of the chest and at the base of the neck, and exfoliation of the epidermis. As the animal did not show any signs of mercurial intoxication and, in particular, no inflammation of the gums or salivation, treatment with biniodide of mercury was continued. The animal became rapidly restored to its normal condition and at the end of the treatment was completely cured of its lymphangitis and its alopecia.

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## REPORTS.

(211) CEYLON. **Administration Reports 1915. Report of Government Veterinary Surgeon.** [STURGESS, G.W.] 8pp. f'cap.

### INFECTIOUS DISEASES.

*Horses.*—One case only of anthrax is reported.

*Cattle*: *Rinderpest.*—There was a considerable decrease in the number of outbreaks during the year. The numbers were as follows:—1,493 cases, number of recoveries 272, number of deaths 1,152, number shot 69. At the close of the year the island was free.

*Foot-and-mouth disease.*—Cases 2,366, recoveries 2,425 (including 128 cases at the end of 1914), deaths 49, balance ill at close of year 20. There was a considerable decrease compared with 1914, when 9,532 cases occurred.

*Surra.*—Bull treated with sodium arsenite, described in report for 1912–1913, remained in good health and no evidence of disease was shown by inoculation of its blood into a rabbit.

*Tuberculosis.*—This disease is rare in native cattle. A country-bred Ayrshire cow from imported parents which was suspected to be affected was tested with tuberculin. A well-marked reaction followed but no traces of tuberculosis could be found on post-mortem examination.

*Anthrax and haemorrhagic septicaemia.*—No serious outbreaks of these diseases occurred during the year.

*Epidemic amongst swine.*—An extensive outbreak of a disease resembling swine fever occurred in the Western Province, of a kind which had not previously been encountered for over 20 years.

Attention was first drawn to this disease by the condition of the carcase of a pig slaughtered at the Colombo abattoir. Its appearance pointed to a disease of the type of an acute haemorrhagic septicaemia. Evidence was obtained that this pig had been brought in from the seat of an extensive outbreak where pigs were dying in some villages by scores. Movement and sale of swine or pork were prohibited in infected villages and Inspectors were authorised to destroy suspected pigs, with the result that the outbreak rapidly subsided. The total number reported was 2,512 cases, 2,414 deaths, 1 recovery, and 97 shot.

The symptoms presented by affected pigs resembled those of acute swine fever in many respects, but the disease appeared to be of a different nature. In no case seen was cough or diarrhoea observed during life, or typical ulcerative lesions in the digestive canal or necrotic areas in the lungs, on post-mortem examination. Inoculation of a healthy young white European pig with 3 cc. of the filtrate from the blood of a dead pig gave a negative result. The disease also appeared in some respects like swine erysipelas, but in no case were the skin lesions characteristic, nor was any valvular complication of the heart noticed. Only in one instance was the heart involved, when the pericardium was found closely adherent to the heart wall.

The general symptoms were dullness, loss of appetite, constipation, catarrhal discharge from the eyes, a more or less diffuse, irregular, purple rash on the skin, and high fever, the temperature becoming subnormal during the later stages of the disease. Before death, giddiness, and tremors, of the head were common. Death usually occurred within a week. The mortality was between 90 and 100 per cent.

On post-mortem examination the lesions were not very pronounced. The heart was normal except in the case mentioned above where the pericardium was adherent. A yellow serous effusion was sometimes present in the pericardial sac. Petechiae were seen on the pleura ; the lungs showed catarrhal pneumonia but were not extensively involved. A yellow serous exudate was generally present in the body cavities. The mucous membrane of the stomach was congested. The intestines showed haemorrhagic spots and ulcerative lesions. The mesentery was usually very congested. The lymphatic glands were as a rule much congested, but in some instances pale and watery. The liver was enlarged and dark in colour. Kidneys congested. Spleen normal.

At the time of the outbreak bubonic plague had appeared and was prevalent in the island. The disease did not appear to be either swine fever or swine erysipelas but an acute septicaemia or toxaemia of the nature of swine plague (*Septicaemia suum* or *Pasteurellosis suum*, *Pneumonie contagieuse du porc* in France, *Schweineseuche* in Germany.) Mr. E. BURGESS, Acting Director of the Bacteriological Institute, made a study of the disease and reference to the organism isolated by him has already been made in this *Bulletin* (Vol. 3, No. 4, Extract No. 256); a Gram-negative diplobacillus (or possibly a diplococcus) was described.

Sturgess has, however, been informed by the Medical Officer of Health, Colombo, who made a bacteriological examination of the first case reported, that bi-polar staining organisms were found in the heart blood, spleen and mesenteric glands. Mice inoculated with a trace of gland substance in sterile salt solution died within 24 hours. Broths inoculated from heart blood showed a pure culture of *Pasteurella suis*. Cultures in MacConkey's sodium taurocholite medium gave negative results using glucose, laevulose, mannite and galactose, while acid reactions were given in parallel cultures with *Bacillus pestis* (human plague). The organism showed bi-polar staining with all basic stains and was Gram-negative.

[The above disease is probably identical with the so-called swine plague described on the Continent of Europe. In England this is considered to be merely one form of swine fever. Further filtration experiments would have been necessary to convince one that the outbreak was not really one of swine fever.—Ed.]

*Cancerous Horn Disease of Cattle.*—Several cases of this disease occurred among dairy cows during the year. The disease is believed to be of a cancerous nature, and for some reason, unexplained at present, the right horn is the one affected. The epithelioma does not develop at the outset but after the horn is shed. The first lesion takes the form of cheesy material which entirely fills the horn. The bony core is entirely obliterated. This appears to be due to bacterial processes of a necrotic nature.

The early signs are a gradual drooping of the affected horn until it is almost down to the ear level. On manipulation it is movable, hot, and somewhat swollen at the base. It is quite soft and can be severed with a knife like cheese. It is not putrid unless an opening has been formed. Commonly, a slight blow while feeding or rubbing detaches it from the skull. The whole cavity is filled with cheesy material which is easily pulled out in masses.

It is then that the serious trouble commences. From one point or another or the marginal area of the skull cavity a malignant growth appears and resists all attempts to heal it. The only chance of successful treatment is amputation in the early stages, as soon as the affected horn is observed to be on a lower level than normal. As a rule early slaughter is advisable before the animal becomes emaciated and the carcase is ruined.

*Biting Flies.*—Specimens of flies causing worry to cattle in the Matale district were identified as *Haematopota singallensis*.

*Sarcoptic Mange in Goats.*—Goats in one district were noticed to be suffering a good deal from mange, which caused emaciation and death of badly infected animals. The presence of a sarcopt was easily detected in skin scrapings. Treatment by dipping in a solution of Izal 1 oz. to 1 gallon of water, followed by the application of a mixture of kerosene, sulphur and cocoanut oil was very effective in eradicating the disease.

(212) Southern Rhodesia. **Report of the Director of Agriculture for the Year 1915.**—22 pp. f'cap. 1916. Salisbury: Govt. Printer.

This includes the Reports of the Chief Veterinary Surgeon (Sinclair, J. M.) and of the Veterinary Bacteriologist (Bevan, Ll. E. W.).

The greater part of the report of the Chief Veterinary Surgeon is devoted to African Coast fever of cattle. The total number of fresh outbreaks and mortality compare unfavourably with previous years, due to the extensive spread of infection in two districts. On the other hand it is recorded with satisfaction that a very large part of the country is now free from this disease.

The policy adopted in dealing with these outbreaks consists in temperature observations and isolation of sick and suspected animals, together with dipping at intervals of three days. When clean veldt is available the herds are moved into it from a temperature camp. A considerable increase in the number of dipping tanks available for the use of farmers has taken place in the course of the year.

Experience points out that the following results usually occur :— (1) Where dipping in solutions of proper strength had been regularly practised prior to an outbreak, the losses were infinitesimal ; (2) where dipping had not been practised and where tanks were not immediately available, the losses were heavy ; (3) where veldt is grossly affected to begin with, cases occur up to twelve months from the institution of regular dipping.

The existence of contagious abortion in cattle was discovered in October 1914, and since then several additional centres of infection have been discovered. The number of abortions observed in every instance was very small and Sinclair concludes that the disease is less virulent than in England because a " fair percentage of calves are born at the proper period." [He is here apparently misinformed, as the same occurs in infected herds in England.—Ed.]

Heavy losses were caused through such diseases as horse sickness, " blue tongue " in sheep, parasitic gastritis of cattle, and in young calves through white scour, etc.

*Report of the Veterinary Bacteriologist.*—No experimental work of interest has been performed. A vaccine for the inoculation of cattle against contagious abortion in cattle consists of an emulsion of the dead organisms. [This was proved to be useless by the Departmental Committee appointed by the Board of Agriculture in England to investigate this disease. Through producing specific agglutinins in inoculated animals, it has further the effect of vitiating the agglutinin test when applied to diagnose the existence, or the spread, of contagious abortion in a herd. The segregation of affected animals is thus rendered impracticable.—Ed.]

(213) Gold Coast. **Report of the Veterinary Department for the Year 1915.** 8 pp. f'cap. 1916. Accra: Government Press.

Nothing of interest, as far as tropical diseases are concerned, is contained in this report. The Veterinary Officer, Mr. W. P. B. Beal, is being temporarily employed by the Veterinary Department of the War Office.

(214) Union of South Africa. **Department of Agriculture. The Third and Fourth Reports of the Director of Veterinary Research. November 1915.**—632 pp, Pretoria: Government Printing & Stationery Office. [Price 3*s.* 6*d.*]

The results of a very large number of well-organised, expensive, and laborious investigations, undertaken by Theiler (Sir A.) and his staff, are detailed in these reports.

Most of the articles have been extracted for this Bulletin; the others are enumerated in the lists of "Recent Literature," not because of inferior value but on account of inability to extract them at present.

Unfortunately a few of the writers in these reports show a tendency to become rather involved in their descriptions. The following passage, for example, taken from Walker's article on Anaplasmosis (p. 511), contains a number of inexcusable errors.

"An animal which had been previously inoculated with blood collected from a beast which was reacting to redwater, and in which redwater parasites were frequent, no apparent reaction to *P. bigeminum*; *P. bigeminum* anaplasmosis or *P. mutans* resulting (see experiment, No. 7.) When reinoculated with blood of another immune animal containing *P. bigeminum* and *P. mutans* infections gave a temperature reaction to redwater, but no redwater parasites were seen in the smears before, during, and after the reaction. (See experiment No. 16.) *P. mutans* appeared from the 54th day."

## Book Review.

(215) Rogers (Thomas B.). [D.V.S.] **Veterinary Handbook & Visiting List.**—119 pp. Demy. 1st Edition. 1916. Philadelphia & London: J. B. Lippincott Company. [Price 6*s.* net.]

In his preface the author makes no claims to originality for the subject matter of his book.

The first nineteen pages contain some very elementary information on bacterial therapy and prescription writing. The great bulk of the book, pages 20 to 107, consists of an enumeration of drugs together with the doses that may be administered to the domesticated animals. The visiting list at the end does not seem to display much advantage over a plain note book.

Probably few practitioners would consider the outlay on this book economical, as similar publications, perhaps of not quite equal value, are supplied to them gratis in this country by several firms of druggists.

## RECENT LITERATURE.

[Continued from this *Bulletin*, Vol. 4, No. 2, pp. 87–88.]

### PROTOZOA.

(216) Carini (A). Ueber die Hundekrankheit Nambi-Uvu und ihren Parasiten, *Rangelia vitalii*. [A Disease of Dogs, Nambi-Uvu and its Parasites, *Rangelia vitalii*.]—*Cent. f. Bakt.* 1. Abt. Orig., 1915. Dec. 29. Vol. 77. No. 3. pp. 265–271. With 2 plates.

(217) Carini (A.) & Maciel (J.). Ueber *Pneumocystis carinii*. [*Pneumocystis carinii*.]—*Cent. f. Bakt.* 1. Abt. Orig., 1915. Sept. Vol. 77. No. 1. pp. 46–50. With 2 plates.

(218) da Cunha (A. M.). Sobre os ciliados intestinaes dos mamiferos II. [The Intestinal Ciliata of Mammals II.]—*Mem. do Inst. Oswaldo Cruz.*, 1915. Vol. 7. No. 2. pp. 139–144. With 1 plate.

(219) Iturbe (J.) & Gonzalez (E.). A New Trypanosoma of the *Vampirops lineatus*.—Pamphlet from Laboratory of Dr. Juan Iturbe, Caracas, Venezuela, 1916. 7 pp. With 1 coloured plate comprising 10 figs.

(220) Johns (F. M.). The Centrifuge Concentration of Malaria Plasmodia for Diagnostic Purposes.—*New Orleans Med. & Surg.* June. Vol. 68. No. 12. pp. 765–768.

(221) King (W. V.). Experiments on the Development of Malaria Parasites in Three American Species of Anopheles.—*Jl. Experim. Med.*, 1916. June. Vol. 23. No. 6. pp. 703–716. With 7 plates.

(222) Lignos (A.). La Leishmaniose canine à Hydra. [Canine Leishmaniosis at Hydra.]—*Bull. Soc. Path. Exot.*, 1916. May. Vol. 9. No. 5. p. 302.

(223) Maggio (C.) & Rosenbusch (F.). Studien über die Chagaskrankheit in Argentinien und die Trypanosomen der "Vinchucas" (Wanzen, *Triatoma infestans* Klug.). ["Chagas" Disease in the Argentine and the Trypanosome of "Vinchucas" (Insects, *Triatoma infestans* Klug.].—*Cent. f. Bakt.* 1. Abt. Orig., 1915. Sept. Vol. 77. No. 1. pp. 40–46. With 2 plates.

(224) da Matta (A.). Sur les leishmanioses tégumentaires. Classification générale des leishmanioses. [Tegumentary Leishmanioses. Classification of Leishmanioses.]—*Bull. Soc. Path. Exot.* 1916. July. Vol. 9. No. 7. pp. 494–503. With 2 plates comprising 12 figs.

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